WATER AND ELECTROLYTE CONTENT AND DISTRIBUTION IN TISSUES OF THERMALLY-ACCLIMATED RAINBOW TROUT, *Salmo gairdneri*

Karen M. Mearow (B.Sc., Hons.)

Department of Biological Sciences

A Thesis

submitted in partial fulfilment

of the requirements for the degree of

Master of Science

September 1978

Brock University

St. Catharines, Ontario

Abstract

The primary objective of this investigation was that of providing a comprehensive tissue-by-tissue assessment of water-electrolyte status in thermally-acclimated rainbow trout, Salmo gairdneri. To this end levels of water and the major ions, sodium, chloride and potassium were evaluated in the plasma, at three skeletal muscle sites, and in cardiac muscle, liver, spleen, gut and brain of animals acclimated to 2°, 10° and 18°C. The occurrence of possible seasonal variations in water-electrolyte balance was evaluated by sampling summer and late fall-early winter populations of trout.

On the basis of values for water and electrolyte content, estimates of extracellular and cellular phase volumes, cellular electrolyte concentrations and Nernst equilibrium potentials were made. Since accurate assessment of the extracellular phase volume is critical in the estimation of cellular electrolyte concentrations and parameters based on assumed cellular ion levels, [^{14}C]-polyethylene glycol-4000, which is assumed to be confined to the extracellular space, was employed to provide comparisons with various ion-defined spaces ($H_2O^{ecs}_{Cl}$, $H_2O^{ecs}_{Cl/K}$ and $H_2O^{ecs}_{Na}$). Subsequently, the ion-defined space yielding the most realistic estimate of extracellular phase volume for each tissue was used in cellular electrolyte calculations.

Water and electrolyte content and distribution varied with temperature. Tissues, such as liver, spleen and brain appeared to be the most thermosensitive, whereas skeletal and cardiac muscle and gut tissue were less influenced. 'Summer' series trout appeared to be more capable of maintaining their water-electrolyte balance than the 'fall-winter' series animals.

The data are discussed in terms of their possible effect on maintenance of appropriate cellular metabolic and electrophysiological functions.

Acknowledgements

I would like to express my appreciation to Dr. A. H. Houston for his guidance and encouragement throughout this investigation.

Dr. S. Pearce provided advice on liquid scintillation procedures.

I would like to thank J. S. Smeda, for all his suggestions and assistance over the past three years, as well as A. M. Calarco, J. Henry and L. McCarty for their contributions to various aspects of the project.

"... all the vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment."

Claude Bernard, 1878.

"In a sense it is stable because it is modifiable--the slight instability is the necessary condition for the true stability of the organism."

Charles Richet, 1900.

Table of Contents

List of Tables

Page

2. Appendix Tables

List of Figures

I. Introduction:

Among most poikilothermic animals, body temperature conforms closely to ambient temperature, and modifications in the latter result in corresponding changes in tissue temperature. This is particularly so in the case of gill-breathing aquatic poikilotherms, since thermal equilibration rates are normally some ten times faster than those seen in terrestrial animals (Hochachka and Somero, 1973).

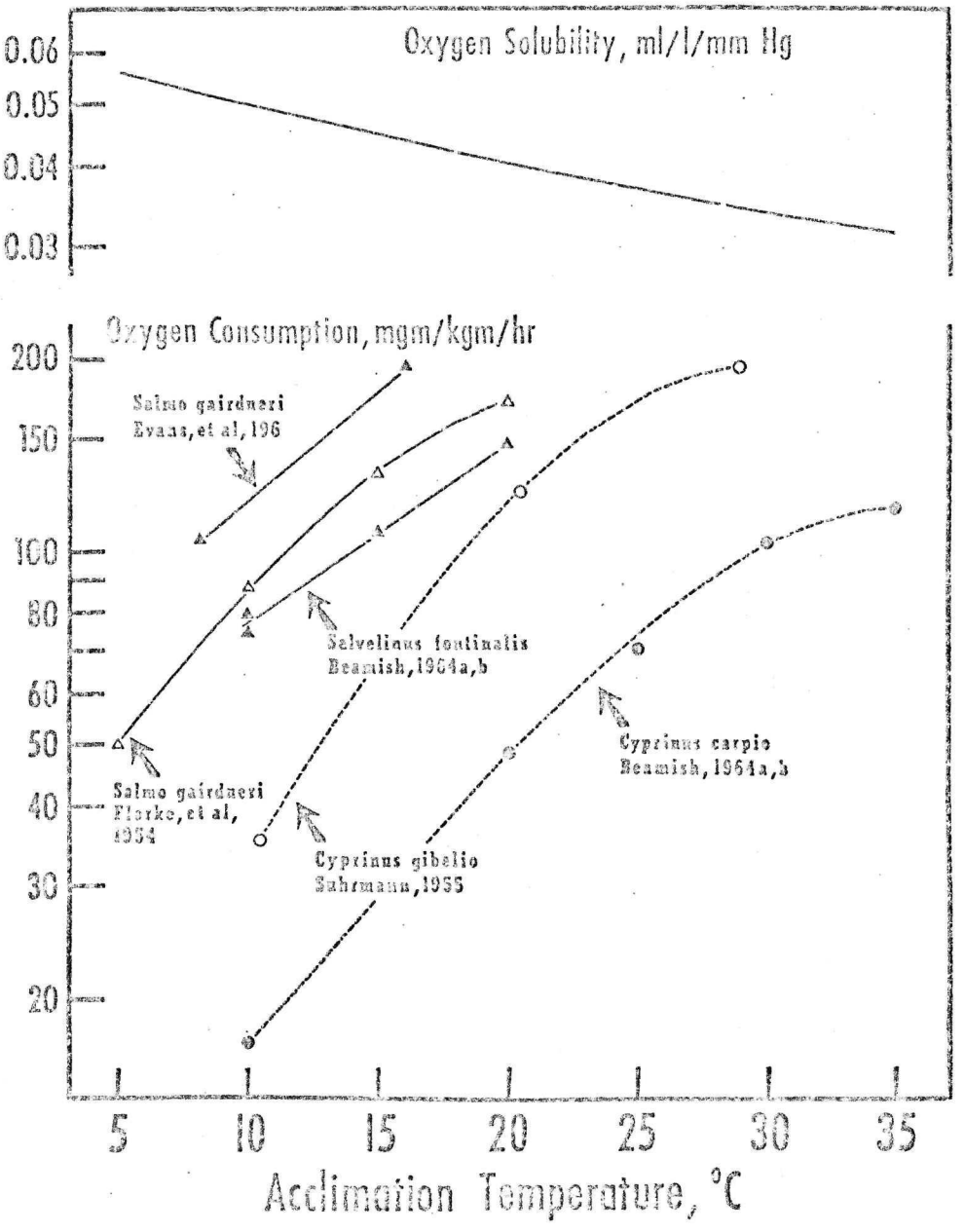
Since most poikilotherms can both survive and function efficiently over relatively wide temperature ranges, it is obvious that some sort of compensation for the effects of temperature change upon physiological and biochemical processes must take place (Hazel and Prosser, 1974).

Several compensatory time courses have been identified (Somero, 1969). Compensation may, for example, be essentially instantaneous, as is the case in some intertidal invertebrates (Hazel and Prosser, 1974). Alternatively it may occur over evolutionary time spans. More commonly, however, individuals respond to changes in environmental temperature over periods of days or weeks. This type of compensation is known as acclimation, if the organism is responding to a single environmental factor under laboratory conditions, or acclimatization if fluctuations in several environmental parameters in the natural environment are involved (Hazel and Prosser, 1974).

Modifications in environmental temperature necessarily lead to changes in metabolism. Temperature effects upon the metabolic rates (as measured by oxygen consumption) of several representative freshwater teleost fishes can be seen in Figure 1. In the rainbow trout, Salmo gairdneri , for example, an increase in environmental temperature from 5° to 20°C is

Figure 1. Oxygen availability and routine oxygen consumption in representative cyprinid and salmonid species following thermal acclimation (taken from Houston,1973).

Fig-1



(from Houston, 1973)

is accompanied by a three-fold increase in standard oxygen consumption (N.B. Standard oxygen consumption refers to standard metabolism, and is an approximation of the minimum metabolic rate in the intact organism (Fry, 1971)). Under the circumstances, oxygen availability decreases as indicated in Figure 1 by the reduction in the Bunsen coefficient of oxygen solubility. The response of the teleost to temperature-related increases in oxygen demand and decreases in availability is complex and includes

(a) elevation of branchial ventilation, perfusion and effective exchange area;

(b) increases in blood oxygen carrying capacity; and

(c) modulation of hemoglobin transport characteristics (Randall et al., 1967; Randall, 1970; Cameron and Davis, 1970).

Increases in cardiac output, ventilatory flow and effective gill surface area impose problems of water-electrolyte balance, since the gills are the principal site of water and electrolyte movements between organism and environment. All will enhance both endosmosis and passive electrolyte efflux. Furthermore, the increases in urine flow rate used as a means of relieving the water-loading problem will inevitably aggravate electrolyte losses.

Despite this, studies carried out to date suggest that most species can maintain fairly stable levels of both water and ions over relatively wide temperature ranges. Some alterations do occur, but these are relatively modest when compared to temperature-related variations in influx and efflux (e.g., Maetz, 1971; Houston, 1973).

However, most of these studies reported to date, have been restricted to consideration of changes in plasma, and in some instances muscle and/or

liver. Little attention has been given to other important tissues. Indeed, thus far there has been no comprehensive assessment of tissue-by-tissue alterations in water-electrolyte balance in any single species of fish. Therefore, the primary objective of this study was that of providing such information. To this end, levels of water and the major ions, sodium, chloride and potassium, were evaluated in the plasma, skeletal muscle, cardiac muscle, brain, liver, gut tract and spleen of rainbow trout, Salmo gairdneri, following acclimation to 2°, 10° and 18°C. These temperatures span the near-lower to near-upper incipient lethal temperature range for this relatively stenothermal, commercially-desireable gamefish species, and also include a mid-range temperature (10°C) widely regarded as optimal for activity, growth and reproduction.

It is also increasingly apparent that osmotic and ionic regulation in the rainbow trout are modified on a seasonal basis (Houston et al., 1968; Murphy and Houston, 1977). Accordingly, the study was extended to provide for sampling of summer and late fall-early winter populations of animals.

Finally, relatively little attention has been given to temperature-related variations in cellular ion levels. This is surprising in view of the growing appreciation of the role played by inorganic electrolytes in modulation of enzyme activities, and therefore, the possible effect which adjustments in cellular ionic composition may have upon the biochemical changes associated with the thermoacclimatory process.

Furthermore, those values for cellular electrolyte concentrations which have been reported are open to question in terms of the methods used in their calculation. Such estimates are ordinarily based upon relationships of the following type:

$$cx = [tx - (ix)(H_2O^{ecs})]/[tH_2O - H_2O^{ecs}]$$

where x = ionic species

H_2O^{ecs} = extracellular volume ($ml \cdot kg^{-1}$)

tH_2O = tissue water ($g \cdot kg^{-1} \cdot ml \cdot kg^{-1}$)

cx = cellular concentration of x

ix = interstitial (≃ plasma) concentration of x

It will be apparent that accurate assessment of extracellular phase volume (H_2O^{ecs}) is critical, for this governs both the estimated cellular volume, [$tH_2O - H_2O^{ecs}$], and the amount of electrolyte assigned to the cellular phase [$tx - (ix)(H_2O^{ecs})$]. Methods for estimation of extracellular phase volume commonly depend on assumed distributions of intrinsic ions (sodium, potassium, chloride) or administered solutes (inulin, sorbitol, iothalamate, polyethylene glycol). It has been frequently suggested (i.e., Lutz, 1972; Schmidt-Nielsen et al., 1972; Beyenbach and Kirschner, 1976) that the latter provide more accurate estimates of the extracellular volume than do the former. However, few comparative studies have actually been carried out, and these have yielded discrepant values. Therefore, as a component of the present study, consideration has been given to the evaluation of extracellular phase volume in terms of tissue [^{14}C]-polyethylene glycol (PEG-4000), chloride, chloride/potassium and sodium 'space', and the effect of spacing technique upon subsequent estimates of cellular potassium, sodium and chloride concentrations.

II. Literature Review:

Literature pertinent to the question of water-electrolyte distribution in freshwater-adapted fishes, and especially the rainbow trout, will be considered under the following main headings:

(1) temperature-related alterations in oxygen requirements as stresses upon ionic balance;

(2) body fluid composition in thermally-acclimated fish;

(3) factors involved in regulation of water-electrolyte balance;

(4) response of metabolic processes to thermal acclimation; and

(5) the role of ions in these processes, with emphasis on their influence upon enzyme activities.

1. Temperature-related alterations in oxygen requirements as stresses on ionic balance

Modifications in environmental temperature necessarily lead to alterations in oxygen demand. As mentioned in the Introduction, increases in temperature are generally accompanied by increases in oxygen consumption. At the same time, the oxygen availability decreases. Responses to these alterations in oxygen demand and availability tend to perturb water and ionic balance, as will be discussed below.

In order to adjust to increases in oxygen requirements, the teleost can

(i) make alterations in branchial exchanger system;

(ii) increase the oxygen carrying capacity of the blood; or

(iii) modify hemoglobin transport characteristics (Randall et al., 1967; Randall, 1970, Cameron and Davis, 1970).

The second and third types of response impose little or no stress on water-electrolyte distribution and will not be considered further, since they are not relevant to this discussion.

Several potentially adjustable factors are involved in the branchial exchanger system. These include ventilatory flow, cardiac output, and effective gill area. In order to keep the amount of oxygen transferred across the gills constant in the face of decreases in oxygen availability as is found with higher temperatures, ventilatory flow and/or cardiac output must be increased (Davis, 1968; Randall, 1968). An increase in the effective branchial exchange area by alteration of blood flow pathways may also occur (Randall, 1970).

However, since the gills are the principal site of water and electrolyte movement, these responses to increased oxygen demand impose stress on water-ionic balance by enhancing endosmosis and passive electrolyte efflux. Randall et al. (1972) have shown that increases in oxygen transfer factors, which result from increases in the functional exchange area of the gills or decrease in diffusion pathlengths between water and blood, lead to increases in ion flux across the gills and especially to increased sodium loss. Water flux has also been shown to increase two-fold with temperature increase of 10°C in goldfish, Carassius auratus (Evans, 1969; Mackay, 1974), and the minnow, Phoxinus phoxinus (Evans, 1969).

Augmented water influx is generally offset by increased urine flow rates at high temperatures (Hickman, 1965; Mackay and Beatty, 1968; Mackay, 1974). Although urinary electrolyte concentrations are reduced, the increase in flow rate leads to net electrolyte loss at higher temperatures (Mackay, 1974).

However, in spite of these problems, studies carried out to date indicate that freshwater teleosts can maintain fairly stable levels of electrolytes and water content over relatively wide temperature ranges. The next element of the review will deal with the pattern of response of the body fluid system of freshwater-adapted fishes to thermal acclimation.

2. Body fluid composition of thermally-acclimated fish

(a) Plasma Electrolytes

Much of the research on freshwater fishes has involved either eurythermal cyprinids, such as carp and goldfish, or the more stenothermal salmonids, a group which includes trout, char and salmon. Fewer have been carried out on other species although some information is available for white sucker, Catostomus commersoni (Hickman, 1964), brown bullheads, Ictalurus nebulosus (Grigg, 1969), freshwater-adapted killifish, Fundulus heteroclitus (Umminger, 1970), and pike, Esox lucius (Soivio and Oikari, 1976).

In general, the plasma of freshwater fish contains high levels of sodium and chloride, and lower concentrations of potassium, calcium and magnesium (Table 1).

Significant differences in patterns of thermo-acclimatory variations in plasma ionic composition have been reported; sometimes for the same, or closely related, species. For example, in the goldfish, Carassius auratus, (Prosser et al., 1970; Mackay, 1974) and carp, Cyprinus carpio (Houston et al., 1970) plasma sodium and chloride did not vary significantly with temperature. Catlett and Millich (1976) however, reported that in goldfish plasma sodium increased with higher temperatures, while chloride remained

Table 1. Plasma Electrolytes: Representative Values in thermally-acclimated freshwater species

Species	Temperature (°C)	Sodium (mM/l)	Chloride (mM/l)	Potassium (mM/l)	
Cyprinus carpio	7 (s)*	128.7 ± 3.2	97.8 ± 3.0	2.73 ± 0.25	Houston et al.,
	2 (s)	124.7 ± 3.0	96.6 ± 2.8	2.52 ± 0.11	1970
	33 (f)*	128.2 ± 1.4	137.0 ± 5.5	2.77 ± 0.13	
	27 (f)	137.3 ± 2.3	122.2 ± 2.6	2.39 ± 0.19	
	17 (f)	130.3 ± 1.4	125.2 ± 1.8	2.93 ± 0.11	
	4 (f)	127.7 ± 1.1	115.6 ± 0.9	2.74 ± 0.13	
Carassius auratus	30	155 ± 8	78.5 ± 1.5	8.3 ± 0.3	Heinicke and
	20	169 ± 9	82.0 ± 0.8	6.1 ± 0.6	Houston, 1965b
	25	165.7 ± 1.9	122.1 ± 1.4	3.26 ± 0.12	Prosser et al., 1970
	15	162.5 ± 2.0	121.5 ± 1.2	3.10 ± 0.13	
	5	154.7 ± 2.1	117.2 ± 1.8	2.38 ± 0.05	
	24	143 ± 2.2	113 ± 0.9	4.2 ± 0.3	Mackay, 1974
	14	147 ± 1.3	117 ± 1.1	3.1 ± 0.1	
	6.5	125 ± 4.7	90 ± 7.4	3.3 ± 0.1	
	30	130 ± 1.3	100 ± 1.2	3.3 ± 0.2	
	20	130 ± 1.4	102 ± 1.1	2.3 ± 0.1	
	10	134 ± 1.3	102 ± 1.0	2.2 ± 0.2	
Salmo gairdneri	16(initial)	161.8 ± 3.06	124.6 ± 1.57	3.81 ± 0.12	Rickman et al., 1964
	16(33 days)	158.4 ± 2.85	135.1 ± 1.47	4.23 ± 0.22	
	6(40 days)	163.1 ± 2.00	134.1 ± 0.41	4.21 ± 0.30	
	21 (s)	145.8 ± 3.1	123.6 ± 3.5	4.85 ± 0.17	Houston et al.,
	21 (w)*	144.2 ± 3.0	127.4 ± 5.0	6.73 ± 0.43	1968
	17 (s)	139.6 ± 3.2	122.2 ± 3.5	5.97 ± 0.20	
	17 (w)	148.7 ± 3.7	128.1 ± 2.7	4.53 ± 0.37	
	14 (s)	144.5 ± 3.9	129.5 ± 3.3	4.61 ± 0.16	
	14 (w)	140.5 ± 3.6	128.1 ± 2.2	3.70 ± 0.16	
	11 (s)	144.9 ± 4.1	127.2 ± 0.8	5.06 ± 0.20	
	11 (w)	146.7 ± 2.7	127.8 ± 1.6	4.78 ± 0.18	
	7 (s)	144.3 ± 3.2	119.0 ± 3.4	4.75 ± 0.15	
	7 (w)	146.7 ± 2.7	127.8 ± 2.2	4.35 ± 0.21	
	3 (s)	141.6 ± 4.2	120.8 ± 2.5	4.42 ± 0.19	
	4 (w)	146.5 ± 2.5	128.6 ± 1.9	2.46 ± 0.12	
	18 (s)	148.4 ± 1.06	120.2 ± 1.26	5.4 ± 0.12	Murphy and Houston,
	18 (w)	153.2 ± 1.48	127.6 ± 1.73	5.3 ± 0.17	1977
	2 (s)	157.8 ± 0.95	129.9 ± 0.98	3.9 ± 0.13	
	2 (w)	158.8 ± 0.85	134.7 ± 1.38	3.9 ± 0.13	
	18 (f)	150.1	131.2	2.9	McCarty and Houston,
	10 (f)	153.7	133.6	1.3	1977
	2 (f)	150.6	132.2	1.5	
Salmo trutta	20 (f-w control)	149.3 ± 1.4	140.5 ± 1.1	5.1 ± 0.5	Gordon, 1959
	10 (f-w control)	145.4 ± 1.1	128.3 ± 1.2	2.8 ± 0.5	
Fundulus heteroclitus	11	162.4 ± 1.6	129.9 ± 2.7	3.9 ± 0.1	Umminger, 1970
	4	127.3 ± 8.5	85.5 ± 5.6	6.9 ± 1.0	
	0.1	113.5 ± 8.3	75.6 ± 10.0	8.1 ± 1.0	
Ictalurus nebulosus	20	163.2 ± 1.5	110.9 ± 1.5	5.1 ± 0.2	Umminger, 1971
	10	161.5 ± 1.4	111.1 ± 1.0	5.7 ± 0.4	
	0.5	100.7 ± 1.6	82.5 ± 6.6	3.2 ± 0.4	

* s = summer; f = fall; w = winter

fairly constant. Murphy and Houston (1974) found that both plasma sodium and chloride of goldfish increased with higher temperatures (20° and 25°C), although at 35°C the concentrations of both electrolytes dropped to the level observed at 5°C.

Brown (Salmo trutta) and rainbow trout (Salmo gairdneri) exhibited significant alterations in sodium and/or chloride concentrations with acclimation (Gordon, 1959; Houston et al., 1968; Murphy and Houston, 1977). The usual response was increases in concentration at higher temperatures. Similar trends are seen in the brown bullhead, Ictalurus nebulosus (Grigg, 1969) and freshwater adapted killifish, Fundulus heteroclitus (Umminger, 1970). On the other hand, McCarty and Houston (1977) found that plasma sodium and chloride in rainbow trout did not vary with temperature, while Hickman et al. (1964) reported that sodium and chloride increased with cold acclimation.

Seasonal variations in one or both of these major plasma electrolytes have also been reported in carp (Houston et al., 1970) and rainbow trout (Houston et al., 1968; Murphy and Houston, 1977).

The less abundant electrolytes, and especially potassium, also show variation in response patterns. In carp and goldfish the most commonly reported situation is increased plasma potassium at higher temperatures (Houston et al., 1970; Prosser et al., 1970). This also occurs in rainbow (McCarty and Houston, 1977; Murphy and Houston, 1977) and brown trout (Gordon, 1959). On the other hand, the absence of significant alterations in potassium have also been reported; e.g., in carp (Houston and Madden, 1968), brown bullhead (Grigg, 1969), and rainbow trout (Hickman et al., 1964).

Magnesium levels are generally thermostable, showing little or no change with temperature (Hickman et al., 1964; Houston et al., 1968; Murphy and Houston, 1977). Calcium concentrations tend to drop slightly at lower temperatures (Hickman et al., 1964; Houston and Madden, 1968; Murphy and Houston, 1977).

(b) Tissue Electrolyte Composition

Changes in the tissue electrolytes of freshwater fishes have not been extensively studied. Only a few tissues have been investigated, and most commonly muscle. Most are characterized by a high potassium content, and lesser amounts of sodium and chloride. Representative values for several tissue types are given in Table 2.

Chloride:

Tissue chloride levels, especially in muscle, tend to be thermostable. Hickman et al. (1964) found no significant changes in the muscle chloride content of rainbow trout held at 6° and 16°C. Similar findings were reported by Toews and Hickman (1969) for this species, and for carp muscle by Houston et al. (1970). Brain chloride content in rainbow trout is elevated at low temperature (Hickman et al., 1964). Liver chloride in winter rainbows decreases with cold acclimation (Murphy and Houston, 1977).

Seasonal variations in the levels at which this electrolyte is regulated are also known to occur. For example, winter rainbow trout muscle and liver exhibit lower chloride levels than summer fish (Murphy and Houston, 1977). Houston et al. (1968) reported that muscle chloride of summer trout was higher than that of fall-winter trout.

Table 2. Tissue Electrolytes: Representative Values in thermally-acclimated freshwater teleosts

Species and tissue	Temperature (°C)	Sodium (mM/l)	Chloride (mM/l)	Potassium (mM/l)	Reference
Salmo gairdneri					
muscle	16	13.8	8.9	87.9	Hickman *et al.*,
	6	9.9	9.01	96.4	1964
brain	16	82.2	47.1	49.0	
	6	60.0	75.5	49.7	
muscle	21 (s)	--	6.4	--	Houston *et al*,
	21 (w)	--	9.5	88.0	1968
	17 (s)	--	7.2	83.0	
	17 (w)	--	10.1	65.0	
	14 (s)	--	6.6	94.0	
	14 (w)	--	9.0	52.0	
	11 (s)	--	6.4	95.0	
	11 (w)	--	10.1	48.0	
	7 (s)	--	7.9	30.0	
	7 (w)	--	9.5	79.0	
skeletal muscle	18 (s)	10.9 ± 0.22	9.1 ± 0.17	128.3 ± 4.23	Murphy and Houston
	18 (w)	12.3 ± 0.23	9.1 ± 0.17	113.0 ± 5.30	1977
	2 (s)	12.8 ± 0.24	9.9 ± 0.21	89.7 ± 4.06	
	2 (w)	11.8 ± 0.32	8.2 ± 0.20	109.6 ± 5.15	
cardiac muscle	18 (s)	30.6 ± 1.23	--	64.5 ± 0.73	
	18 (w)	26.8 ± 0.55	--	66.2 ± 0.96	
	2 (s)	23.8 ± 0.80	--	62.2 ± 0.82	
	2 (w)	23.5 ± 0.77	--	64.7 ± 0.84	
liver	18 (s)	34.4 ± 1.20	41.9 ± 0.92	99.0 ± 3.20	
	18 (w)	36.4 ± 0.72	45.0 ± 0.81	97.5 ± 1.20	
	2 (s)	29.1 ± 0.90	43.3 ± 0.56	108.6 ± 1.09	
	2 (w)	25.7 ± 0.60	37.3 ± 1.10	105.3 ± 1.80	
Salmo trutta					
muscle	20	12.0 ± 1.3	9.6 ± 0.6	142.5 ± 1.7	Gordon, 1959
	10	8.6 ± 2.0	8.8 ± 1.5	136.8 ± 2.5	

Sodium:

In rainbow trout, skeletal muscle sodium levels are also relatively thermostable (Hickman et al., 1964; Toews and Hickman, 1969; Murphy and Houston, 1977). Cardiac muscle in this species, however, exhibits significant increases in sodium content at higher acclimation temperatures, and this is true of liver as well (Murphy and Houston, 1977). Prosser et al. (1970) reported that goldfish muscle sodium rose between 5° and 25°C, with maximum values found at 15°C.

Sodium levels have also been reported to vary on a seasonal basis. For example, in summer rainbow trout cardiac muscle and liver have a higher sodium content than winter trout (Murphy and Houston, 1977). Gordon (1959) reported the sodium content of fall brown trout muscle to be higher than that of summer fish.

Potassium:

Trout muscle and brain potassium levels increased upon cold acclimation (Hickman et al., 1964), and similar variations were observed in trout liver (Murphy and Houston, 1977), and in carp muscle (Houston et al., 1970). On the other hand, Murphy and Houston (1977) found no variation in the potassium content of skeletal and cardiac muscle, under 'winter' and 'summer' photoperiod-temperature conditions. As was the case with sodium and chloride, seasonal changes in potassium levels have also been observed. For example, brown trout sampled in fall had higher muscle potassium levels than those sampled in the summer (Gordon, 1959). On the other hand, summer sampled and summer-photoperiod trout exhibit higher muscle potassium content than fall fish (Houston et al., 1968; Murphy and Houston, 1977).

(c) Water Content

Several studies have related water content and distribution to acclimation temperature. Most deal with individual tissues, and particularly muscle and liver, and not all are in agreement. Goldfish, for example, have been reported to show no variation in muscle water content with temperature (Das, 1967; Heinicke and Houston, 1965a; Prosser *et al.*, 1970). In fall-sampled carp and rainbow trout muscle water content decreased at warmer acclimation temperatures (Hickman *et al.*, 1964; Houston *et al.*, 1968; Murphy and Houston, 1977). The water content of liver, however, typically increases with temperature (Kanungo and Prosser, 1959; Das, 1967; Parvatheswararao, 1967; Murphy and Houston, 1977). Hickman *et al.* (1964) also found no significant variation in the brain water content of rainbow trout with temperature. That of *Etropeus maculatus*, however, declined at reduced temperatures (Parvatheswararao, 1967).

Goldfish plasma water levels tended to increase in the cold (Catlett and Millich, 1976). No change was observed in that of rainbow trout by Hickman *et al.* (1964) or Toews and Hickman (1969).

It is difficult to generalize on changes in water content with temperature. Tissues from the same animals do not respond in the same manner, but decreases in muscle water and increases in liver water content tend to be associated with acclimation to higher temperatures.

(d) Water Distribution

In those instances in which tissue water content changes with acclimation, it is of some importance, because of the effect upon cell ion concentration, to ascertain whether distribution between cellular and extracellular phases has been altered.

As noted in the Introduction, assessments of water distribution are based upon estimates of extracellular phase volume. These, in turn, are usually based upon the calculated volumes of distribution of either intrinsic or extrinsic solutes. Chloride and sodium 'spaces', for example, are calculated on the basis of plasma and tissue levels of these ions and assume that these ions are confined to the extracellular space (Manery, 1954). Since small quantities of each are found in cells (Manery, 1954; Conway, 1957; Daniel, 1958; Gordon, 1959), calculated 'spaces' slightly exceed the true extracellular phase volume. The chloride/potassium space calculation developed by Conway (1957) assumes that chloride and potassium are freely distributed between the cellular and extracellular phases in accordance with a Donnan equilibrium (i.e., $K^+_e Cl^-_e = K^+_i Cl^-_i$).

Several studies have investigated the effects of temperature on water distribution. In a number of cases, the extracellular phase volume (ECPV) appears to be little influenced by temperature. For instance, Gordon (1959) reported no significant variation in Cl^- space of brown trout held at 10° and 20°C. Similar findings for Cl^- and Cl/K space of rainbow trout muscle held at 6° and 16°C were reported by Hickman et al. (1964). Brain Cl^- and Cl/K spaces, however, increased with cold acclimation in the same study. The Cl^- space of goldfish muscle (Heinicke and Houston, 1965b) and carp muscle (Houston et al., 1970) showed no significant temperature-related modifications. The Cl/K space of rainbow trout muscle also exhibited no variation with temperature although the Cl/K space of winter trout liver was larger at higher temperatures (Murphy and Houston, 1977).

Estimates of ECPV based on the sodium space are also available. Hickman et al. (1964) reported that sodium space of rainbow trout muscle

and brain decreased with cold acclimation, while Toews and Hickman (1969) observed a decline in muscle sodium space in the same species.

Cellular phase volume, which is calculated from the difference between tissue water and ECPV, appears to either vary little with temperature, or to exhibit some decrease at higher temperatures. For example, the cellular phase of fall rainbow trout muscle decreased at high acclimation temperatures, while that of summer fish showed no variation (Houston et al., 1968). The same pattern was reported for both muscle and liver of the same species by Murphy and Houston (1977).

In spite of the sparseness of data on this matter, it seems that extracellular phase volume either remains stable or rises with increased temperature, while cellular phase volume estimates indicate decreased volume at higher temperatures.

(e) Cellular Ion Levels

Gordon (1959) calculated cellular sodium in muscle of brown trout and found that the levels were both low and thermostable. Cellular sodium of rainbow trout muscle decreased with cold acclimation. Muscle cellular potassium did not change, while brain cellular potassium increased with cold acclimation (Hickman et al., 1964). Muscle cellular sodium and chloride in carp tended to rise with warm acclimation, while cellular potassium tended to decrease under similar conditions (Houston et al., 1970). Murphy and Houston (1977) reported that cellular potassium concentration in muscle increased while that in liver decreased with increases in temperature. Cellular magnesium responded in a similar manner, while calcium remained stable. Cellular electrolyte levels tend to follow quite closely the pattern exhibited by the tissue ionic content in response to differing temperatures (Hickman et al., 1964; Murphy and Houston, 1977).

The factors involved in maintenance of body fluid composition will now be considered.

3. Factors involved in the regulation of water-electrolyte balance

From the previous sections, it will be clear that teleost fish are capable of compensating for thermal effects. Changes in body fluid composition are much less than would be expected as a result of observed flux rates (Evans, 1969; Houston, 1973; Mackay, 1974).

The control of water-electrolyte balance involves a variety of mechanisms governing transfer and exchange processes sited at the boundaries at which movements between the organism and its environment, and between various compartments within the body fluid system can occur (Houston, 1973). The former include the gills, kidney and, to a lesser extent, the gut tract. These will be discussed in turn.

In freshwater fishes, the main function of the kidney lies in the elimination of excess water. Large volumes of urine are formed from which filtered electrolytes are reabsorbed, and consistent with this, the nephrons of these animals are characterized by low tubular water permeability and vigorous monovalent ion reabsorption systems (Hickman and Trump, 1969). As might be anticipated, the increased water influx associated with exposure to higher temperatures is countered by substantial increases in glomerular filtration rates which lead to amplified urine flows. In a variety of species including carp (Pora and Prekup, 1960; Houston, 1973); white sucker (Hickman, 1965; Mackay and Beatty, 1968) and goldfish (Mackay, 1974), the Q_{10} for urine flow is in excess of 2.0. This is believed to

result largely from increases in numbers of functional glomeruli rather than increased flow per nephron (Mackay and Beatty, 1968). Since there is no increase in water reabsorption, tubular water permeability appears to be independent of temperature (Hickman and Trump, 1969). Although ion transport in the kidney is not as well understood as that in the gill, (Na^+/K^+)-ATPase and carbonic anhydrase are known to be present and necessary for ion reabsorption (Hickman and Trump, 1969; McCartney, 1976; McCarty and Houston, 1977). Inhibition of carbonic anhydrase with acetazolamide, inhibits salt recovery from the urine (Maren, 1967). Na^+/K^+-ATPase is involved in the active absorption of sodium. An unknown component of chloride reabsorption is thought to occur passively in accompaniment with Na^+. Potassium can undergo either net secretion or net reabsorption against its concentration gradient (Hickman and Trump, 1969).

Although large amounts of water can be excreted as a dilute urine, and much of the electrolytes reabsorbed, urinary electrolyte losses increase substantially at higher temperatures. In goldfish, for example, despite reductions in urinary electrolyte concentration, all ions other than potassium are lost more rapidly at high temperature (Mackay, 1974). This is true of carp as well (Houston, 1973).

Thus, although control of body water levels seems to be effectively carried out by the kidney, it is apparent that control of electrolyte levels must involve extrarenal processes.

In order to compensate for temperature-related elevations of ventilatory rates and associated water fluxes which lead to increased branchial electrolyte loss, there must be a mechanism or mechanisms operating to limit this loss and/or recruit additional electrolytes from the environment. For

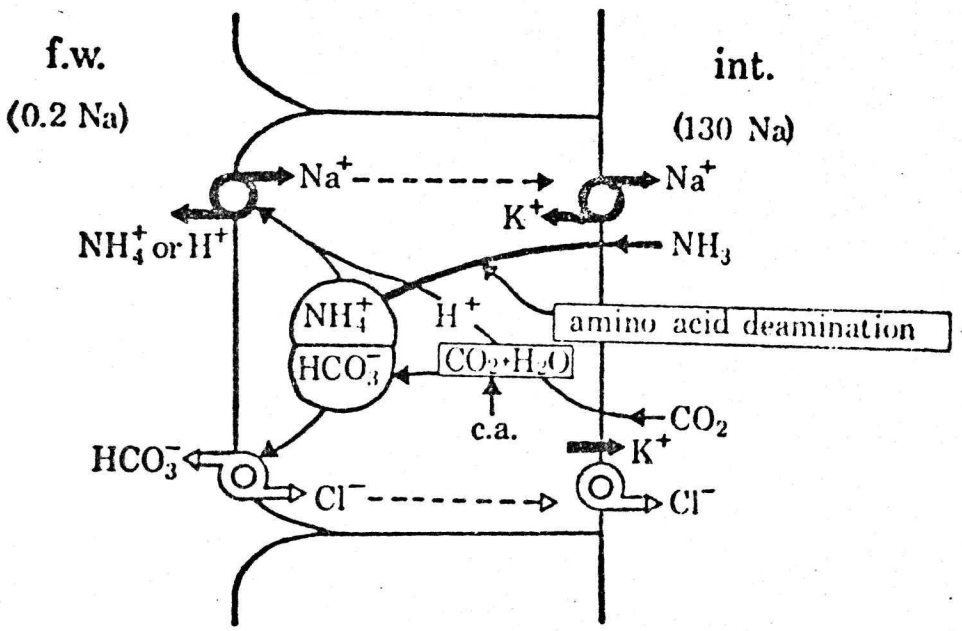
example, there is evidence that branchial ionic permeabilities are reduced at high temperatures, since electrolyte fluxes are below what would be expected from corresponding changes in water influx (Maetz, 1972; Cameron, 1976). In addition, absorption processes located on the lamellar epithelium, and, more specifically, in the 'chloride' cells of the gill have been implicated as sites high in ion transport activity and transport enzymes (Maetz, 1971, 1974).

Although branchial electrolyte absorption is generally regarded as an active process, many of the steps involve passive exchanges, (Maetz, 1971), which allow the animals to offset some of the metabolic costs of ionic regulation (Houston, 1973). Maetz's widely accepted model of branchial ion uptake involves counter-ion exchange for both Na^+ and Cl^- in order to maintain electroneutrality, and is illustrated in Figure 2. NH_4^+, formed from ammonia in the liver and kidney, or H^+, liberated from $CO_2 + H_2O$, are exchanged for Na^+. The Cl^- exchange mechanism involves HCO_3^- as the counterion, liberated from hydrolysis of CO_2. In addition, a Na^+/K^+ ATPase, thought to be located on the serosal side is believed to pump Na^+ out and K^+ in to make up for the K^+ lost down the concentration gradient. The types of exchange suggested have been demonstrated in goldfish (Maetz and Garcia-Romeu, 1964; DeRenzis and Maetz, 1973) and in trout (Kerstetter and Kirschner, 1972) and Arctic grayling, Thymallus arcticus (Cameron, 1976).

The response of these systems to temperature has also been investigated. For example, Cameron, (1976), has shown that the rates of both branchial $Na^+/H^+(NH_4^+)$ exchange and Cl^-/HCO_3^- exchange in Arctic grayling rise with increased acclimation temperature. It has been suggested that thermal acclimation is associated with adjustments in HCO_3^- and NH_4^+ concentrations

Figure 2 Functional model of the 'chloride cell' in freshwater. Independent Na^+/NH_4^+ or H^+ and Cl^-/HCO_3^- exchanges are located on the mucosal border. The role of carbonic anhydrase (c.a.) and of deaminating enzymes in the production of HCO_3^-, H^+ and NH_3 is also shown. On the inner border, a Na^+/K^+ exchange and a Cl^- pump are depicted. (from Maetz, 1971).

Fig. 2



(FROM MAETZ, 1971)

favoring increased exchange at high temperatures (Maetz, 1972; Powers, 1974; Cameron, 1976).

Active transport enzyme systems, such as Na^+/K^+ stimulated, and anion (HCO_3^-)-stimulated ATPase, are also highly temperature sensitive. They tend to be inhibited in the cold (Giles and Vanstone, 1976) and to exhibit increased activity in warm temperatures (Murphy and Houston, 1974; McCarty and Houston, 1977). It appears that ATPase is not of prime importance in cold-acclimated animals, and that renal electrolyte recovery may be sufficient for maintenance of ion levels (Jampol and Epstein, 1970). At warmer temperatures, the enzyme activities increase, as might be expected, to aid in electrolyte recruitment.

At the same time, the carbonic anhydrase system has a relatively low thermal sensitivity (Davis, 1961). McCarty and Houston (1977) and Houston and Mearow (1978, unpublished observations) have shown that branchial carbonic anhydrase activity is relatively high and thermostable. This system might maintain sodium uptake in the cold-acclimated fish, by fuelling the Na^+/H^+ exchange, when the ATPase system is relatively inactive. At warmer acclimation temperatures, erythrocytic carbonic anhydrase activity is increased (Smeda and Houston, 1978), and along with the enhanced ATPase activity likely provides for the enhancement of electrolyte uptake at required at elevated temperatures.

In addition, similar mechanisms may be operating between the various body compartments and cellular phases to maintain tissue and cellular electrolyte concentrations and distribution.

4. Response of metabolic processes to thermal acclimation

Although abrupt changes in environmental temperature may initially prompt marked alterations in the metabolism of poikilotherms, acclimation normally leads to some form of compensatory response (see reviews by Hochachka and Somero, 1973; Hazel and Prosser, 1974).

The reaction pathways contributing to overall metabolism are often highly temperature sensitive. Since metabolism can be regarded as the sum of all biochemical reactions, and since virtually all of these are enzyme catalyzed, Hochachka and Somero (1973) suggest that compensatory metabolic response is essentially a question of the control of rates and types of enzymatic activity.

A review of pertinent literature (Hochachka, 1967; Hochachka, 1971; Fry and Hochachka, 1970) leads to the conclusion that metabolism is fundamentally reorganized during thermal acclimation so as to minimize the effects of direct temperature effects upon rate functions. Several examples of this have been documented. In the tissues of cold-acclimated trout, the rate of glycolysis is increased five times by comparison with that of warm-acclimated fish (Hochachka and Hayes, 1962). Species differences in response are, however, common. For example, muscle from cold-acclimated green sunfish, Lepomis cyanellus, showed decreased glycolysis, while that of brain was increased (Shaklee et al., 1977). Goldfish, Carassius auratus, exhibited no change in glycolysis with temperature, but did display increased oxidative phosphorylation activity in the cold (Wilson et al., 1975), as did muscle from cold-acclimated sunfish (Shaklee et al., 1977) and rainbow trout (Dean, 1969). Similar results have been obtained with freshwater-adapted killifish, Fundulus heterclitus (Bolaffi and Booke, 1974).

Pentose shunt participation in glucose metabolism is also altered during acclimation. Kanungo and Prosser (1959) found that stimulation of respiration following cold-acclimation of goldfish was largely due to increased pentose shunt activity. In addition, Shaklee et al. (1977) found that the activity of tricarboxylic acid cycle and electron transport chain enzymes increased significantly in cold-acclimated green sunfish. This has also been observed in cold-acclimated trout (Dean, 1969).

Protein synthesis rates are also generally higher during cold acclimation (Das and Prosser, 1967). Lipid metabolism, too, is affected by temperature with cold temperatures causing increases in the degree of unsaturation of fatty acids, and changes in the lipid composition of various tissues (Knipprath and Mead, 1968; Roots, 1968). Since unsaturated lipids would tend to maintain membrane 'fluidity' in the face of decreasing temperatures, and since many enzymes are membrane-bound and require certain freedom of movement, changes in lipid composition may be important in the maintenance of certain enzymatic activity at cold temperatures.

The preceding are general observations drawn from the literature. It can be seen that alterations do occur in metabolic processes in response to temperature acclimation, and that these adjustments are tissue-specific, as well. However, the significance of such metabolic reorganization still remains unexplained.

5. Ions and their influence upon enzymatic activity

This section will deal with the role that cellular electrolytes play in control or regulation of metabolic processes, with emphasis on their influence upon enzyme activity.

Cellular electrolytes play a major role in the regulation of metabolic activity, through their modulation of enzyme activities (Bygrave, 1967; Hazel and Prosser, 1974). Magnesium and/or potassium, for example, frequently stimulate enzyme activities, while calcium and/or sodium act as inhibitors (Bygrave, 1967). Temperature-related variations in ionic concentrations as are known to occur during thermal acclimation of poikilotherms (reviewed by Houston, 1973) may be of some importance in this regard.

Divalent cation concentrations appear to be more sensitive to thermal conditions than are those of the major monovalent ions (Hazel and Prosser, 1974). Since magnesium and calcium are particularly potent stimulators and inhibitors of enzyme action, their relative concentrations may well determine the relative activities of key enzymes, and if these are located at metabolic branch points, govern carbon flow through alternative pathways.

In addition, of course, temperature effects on metabolic paths may stem from direct thermal effects upon enzymes, or from the regulation of catalytic function by allosteric interactions or by alterations in enzyme-substrate saturation levels (Hochachka and Somero, 1971, 1973). The importance of ionic modulation in relation to other influencing factors has, however, been difficult to assess. As noted in the Introduction, there is relatively little information available regarding cell ion levels. Furthermore, few studies have been reported in which ionic effects have been examined under physiologically-realistic circumstances.

There are, however, several studies which define the roles of selected ions. Bygrave (1967) outlines several examples in his review. For instance, the phosphate transferring enzymes involved in transfers of phosphate from ATP to ADP or AMP appear to attain maximal activity only

when a divalent cation, magnesium, is present. Bygrave suggests that magnesium is critical in the formation of a complex of the enzyme and adenine nucleotide. Calcium competitively antagonizes this action through formation of an inactive complex.

Relatively few studies involving fishes have been reported. Nevertheless, those available tend to support the contention that inorganic electrolytes are vital in metabolic regulation, and thus point to the importance of the ionoregulatory features of thermal acclimation.

Some examples dealing with intermediary metabolism and with ion transport will next be presented in support of this contention. Somero and Hochachka (1968), for example, investigated the pyruvate kinases of rainbow trout and an arctic species, Trematomus bernacchi. This enzyme is activated by potassium, but to a lesser extent than is the case in mammals. They suggest that reduced potassium dependence may be significant since intracellular potassium concentrations in trout muscle are decreased at lower temperatures. The properties of dehydrogenases from another arctic species, the yellowfin sole, Limanda aspera, were investigated by Behrisch (1972). Potassium and sodium were shown to be particularly effective modulators of dehydrogenase activity. Their effectiveness was, however, conditioned by temperature conditions and substrate and cofactor concentrations. Sodium stimulated two pentose-shunt enzymes, glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase, equally well at all temperatures, but as substrate concentrations decreased, sodium stimulation increased. A glycolytic enzyme, 3-phosphoglyceraldehyde dehydrogenase, from the sole, was also activated by sodium at all temperatures. Potassium, however, acted as an activator at low temperatures, and as an inhibitor at high temperatures. Behrisch (1972) also observed that the maximum substrate

affinities of the enzymes tested occurred at temperatures close to the acclimatization temperature of the specimens used, and that potassium appeared to be involved in this phenomenon. It is clear from this study that both sodium and potassium are important in regulating enzyme activity, and that their effects are variable in relation to temperature.

The major site of ion movement into and out of the fish is, as previously noted, the gills. The total gill surface area in contact with the medium is in excess of ten times that of all other external surfaces combined. The major enzymes associated with ion movements are currently believed to be (Na^+-K^+)-stimulated Mg^{2+}-dependent ATPase (E.C. 3.6.1.3; ATP phosphohydrolase) and carbonic anhydrase (E.C. 4.2.1.1, carbonate hydrolyase) (Maetz, 1971). The activities of both are governed by the presence, and relative amounts of various ions. (Na^+/K^+)-stimulated ATPase, of course, requires sodium and potassium, as well as magnesium and ATP, in order to function. Its activity depends upon the concentration of sodium and potassium present, the ratio of the two and also ionic strength (Johnson et al., 1977). (Na^+/K^+)-ATPase is envisaged as a protein dimer embedded in the membrane phospholipid bilayer, which uses energy generated by the hydrolysis of ATP to move ions across the membrane (Towle et al., 1977). There is much controversy with respect to the mechanism prompting ion translocation during the course of hydrolysis. Yager (1977) discusses several current models. Among these is a two-site model, in which Na^+ and K^+ are believed to bind simultaneously to two sites in the 'pump'. These are reversed within the protein, and the ions released into the opposite sides during the hydrolysis of ATP. Another model visualizes only a single cation site. This assumes that two conformational states exist. In state

I, Na^+ is bound on the cytoplasmic side. In state II, K^+ is bound on the medium side. State II, which is considered to be a phosphorylated intermediate, is dependent on Na^+ for formation, while K^+ is necessary for its breakdown.

Carbonic anhydrase catalyzes reversibly the hydration of carbon dioxide to H^+ and HCO_3^- and has been implicated in ion transport, particularly in the gill (Maetz, 1971). The enzyme is considered to be a monomeric protein with a requirement for Zn^{2+} as a cofactor. Halide ions are known to inhibit carbonic anhydrase (Bundy, 1977).

Although ions clearly influence enzyme activities *in vitro*, the extent to which they affect enzymic reactions and alter metabolism *in vivo* during thermal acclimation remains uncertain. Such control will most likely prove to be very complex, since to be effective they must be available in the non-complexed state and appropriately distributed. Moreover, many enzymes have more than one activation site and are subject to modulation by agents other than inorganic electrolytes. Nevertheless, existing evidence suggests that even relatively small changes in either electrolyte levels or electrolyte ratios can lead to significant modification in enzyme function.

III. Methods:

1. Source and Maintenance of Experimental Stock

Rainbow trout, Salmo gairdneri, were obtained from a local supplier (Goosen's Trout Farm, Otterville, Ontario). (See Appendix I, for a review of Natural History of rainbow trout).

Three batches of trout from the same stock were used. Fish obtained in late spring of 1975, and sampled during the summer of 1975 are termed 'summer' fish. A second group received in the early fall of 1975, and sampled in late fall of 1975 are termed 'fall' fish. The final batch of trout were obtained in late fall of 1976, and sampled in the late spring of 1977 in conjunction with the space evaluation study.

Upon arrival, fish were inspected, separated into three groups and placed in constant-flow 500 l fibreglass tanks (Frigid Units Inc., Toledo, Ohio, LS-700). These were equipped with model BHL-1076 recirculating refrigeration units (Frigid Units Inc.) and with 1000 watt stainless steel heating coils. Water temperature was regulated to within ±0.5°C of the set-point via water temperature sensors linked to locally-designed and constructed controller units (J. Rustenberg, unpublished). In addition, supplementary dechlorinated water inflows provided full exchange of tank water three times daily. Dissolved oxygen levels were normally 80% of saturation or better, although oxygen content per se was reduced at higher acclimation temperatures.

Fish were initially held at hatchery water temperature (8-10°C) until resumption of normal feeding, and other activities. Water temperature was then changed by about 1.0°C every other day until acclimation temperatures of 2°, 10° and 18°C were attained. They were then allowed to acclimate for

for a minimum of 4 weeks prior to sampling. This time period was chosen on the basis of thermal shock experiments (Heinicke and Houston, 1965b; Reaves *et al.*, 1968) and experiments following the time course of thermal acclimation (Sidell *et al.*, 1973) which suggested that following temperature shocks or changes, a new steady state is reached in three to four weeks. A 12-hour light/12 hour dark photoperiod cycle was maintained throughout all experiments.

All fish were fed daily, *ad libitum*, on a commercial preparation (Purina Trout Chow, Purina Industries). From their activity and outward appearance the fish seemed healthy and no mortalities due to any specific disease were observed.

The tanks were also equipped with polyethylene foam filters, which were removed and cleaned on alternate days. Fecal material and unused food were removed daily.

2. Sampling Procedure

(a) Blood Samples

Trout were taken by use of a dip net. Chemical anesthetization was not used in the thermoacclimatory experiments because of the significant effects of this upon plasma electrolyte levels (Houston *et al.*, 1971). It was however, necessary to use anaesthesia in the space evaluation study outlined below.

Individuals were stunned by a blow to the head, blood drawn by caudal puncture into ammonium heparinized (Sigma Chemical Co., St. Louis, Mo., 50,000 units) syringes, and centrifuged in a Fisher micro-centrifuge at 5,000 G for 5 minutes.

Plasma was immediately removed using a capillary pipette, placed into a small plastic (polystyrene) tube, sealed, labelled and stored at -80°C until analyses could be carried out. Erythrocyte fractions were discarded.

The weight, length and sex of each specimen were recorded.

Fish were then killed by severing the spinal cord, and tissue samples removed.

(b) Tissue Samples

Love (1970) has provided evidence of substantial regional variation in composition along the epaxial muscle band. Therefore, three epaxial muscle sites were sampled from the left side of the fish:

(i) post-opercular muscle--excised from the muscle band just posterior to the operculum;

(ii) mid-dorsal muscle--sampled from just below the dorsal fin; and

(iii) caudal muscle--removed from the tail area below the adipose fin.

Liver samples were removed from the posterior lobe of that organ. The heart was dissected free of conal tissue, divided in half and blotted free of excess blood. The brain was removed intact, and the well-vascularized hypophysis dissected away. A 20-30 mm segment of the posterior portion of the intestine was removed, flushed and blotted. The spleen was removed intact.

All tissue samples were dissected free of any attached skin, scales, connective tissue and/or fat, lightly blotted on absorbent tissue, wrapped in a double layer of Parafilm (American Can Co., Dixie/Marathon, Greenwich Ct) and stored at -80°C prior to use.

3. Sample Preparation for Analyses

Where sample sizes permitted, all analyses were performed in duplicate. Frozen tissues were thawed in weighed test tubes, and the tissue wet weight recorded. They were then dehydrated for 24 hours at 105°C, and tissue water content calculated from differences in wet and dry tissue weight (g H_2O/kg wet weight ≃ ml/kg).

Dried tissues were extracted in 5.0 ml of 0.1 N HNO_3 for 24 hours with periodic shaking (Little, 1964). This 0.1 N nitric acid digest was chosen since it allowed for analysis of all three ions (Na^+, K^+ and Cl^-) on one tissue sample, a factor of some importance since many samples, especially heart and brain were too small to permit use of two procedures.

(a) Chloride Analysis

Tissue and plasma chloride determinations were carried out using a Buchler-Cotlove chloridometer (Buchler Instruments Inc., Fort Lee, N. J.). Samples and standards were prepared in duplicate. A 1.0 ml aliquot of tissue extract was pipetted into a vial containing 2.0 ml of the reagent solution (0.1 N HNO_3 + 10% acetic acid). For plasma a 10.0 µl volume of sample was used. Immediately prior to determination, 3 drops of gelatin reagent (6.2 g of 60:1:1 gelatin:thymolblue:thymol/1 H_2O) were added to each vial. Standards were prepared in a similar manner. Versatol (General Diagnostics, Morris Plains, N. J.), a standardized human reference serum containing 103 mM/1 Cl^-, was used for comparison with plasma samples. For tissues, a standard containing 4 µmoles of NaCl/ml was employed. Blank solutions containing all reagents, plus 10 µl of distilled water for plasma analyses, or 1.0 ml of 0.1 N HNO_3 for tissue analyses, were also prepared.

These were run first to provide a stable 'blank' time reading.

Samples and standards were titrated by the release of Ag^{+2} ions from the anode into solution, and the simultaneous measurement of solution conductivity. A $AgCl_2$ complex is formed, and the titration reaches end-point when all Cl^- ions in solution have been complexed with Ag^{+2}. The time taken to reach end point is linearly related to chloride concentration, and can be converted to chloride concentration by the following formulae:

$$\text{Plasma } Cl^- \text{ (mM/l)} = \frac{T_S - T_B \cdot [\text{standard}]}{T_{STD} - T_B}$$

$$\text{Tissue } Cl^- \text{ (mM/kg)} = \frac{T_S - T_B \cdot [\text{standard}]}{(T_{STD} - T_B)(t_{wt})}$$

where T_S = sample titration time

T_B = blank titration time

T_{STD} = standard titration time

[standard] = concentration of standard

t_{wt} = tissue wet weight in kg

(b) Sodium and Potassium Analyses

Tissue samples were prepared for cation analyses by adding 500 µl of tissue extract to 10.0 ml of 7.987 g $SrCl_2 \cdot 6H_2O$/1 H_2O solution (0.25% strontium solution). Plasma samples were prepared by adding 200 µl to a 7.606 g $SrCl_2 \cdot 6H_2O$/1 H_2O (0.25% strontium) solution. This diluent was used to avoid any interference from sulphates and phosphates (Paschen and Fuchs, 1971). A series of standards containing appropriate amounts of Na^+, K^+, Ca^{+2} and Mg^{+2} (in the same proportions as expected in the samples) were

prepared using BDH (British Drug House Chemicals, Toronto, Ont.) Atomic Absorption single standards, each containing 1000 ppm of the respective ions. Mixed, rather than single standards were used. It has been shown that determination of K^+ in the presence of excess Na^+ (as is the case in plasma) results in higher K^+ values than if K^+ is determined alone (Ramirez-Munoz, 1968; Byrne, et al., 1972). K^+ does not appear to have this effect on Na^+ determinations. The standards were used to construct a calibration curve from which values of unknowns could be estimated.

Determinations were made with a Unicam SP-90 spectrophotometer used in the emission mode, and optical density readings recorded on a Fisher Recordall Series 5000 recorder. The instrument was blanked with a solution of $SrCl_2 \cdot 6H_2O$ containing 0.25% strontium and a series of five standards for the appropriate samples, were run between each set of 10 samples to check for drift. This sequence was followed for all samples. Plasma Na^+ and K^+ values were then estimated directly from the calibration curve. Tissue Na^+ and K^+ were calculated using the following formulae:

$$\text{X ppm/kg} = \frac{(\text{X ppm})(\text{dilution factor})(5 + \text{T})}{(\text{T})}$$

$$\text{X mM/kg} = \frac{\text{ppm/kg}}{\text{X molecular weight}}$$

where X ppm = parts per million values estimated from calibration curve for the appropriate cation, either Na^+ or K^+

T = tissue wet weight in kg

4. Estimation of Extracellular Phase Volume

(a) Catheterization

[^{14}C]-polyethylene glycol ([^{14}C]-PEG-4000) is widely regarded as a more reliable extracellular marker than either labelled inulin or iothalamate (glofil) (Schmidt-Nielsen et al., 1972; Schmidt-Nielsen and Renfro, 1975; Beyenbach and Kirschner, 1976), and was used as a basis for comparison with extracellular phase volume defined in terms of intrinsic electrolytes, i.e., Cl^-, Cl/K and Na^+ spaces.

Rainbow trout acclimated to 10°C were used in this phase of the study, and were maintained under the conditions previously described. Fish were netted, and immediately placed in freshly prepared tricaine methanesulfonate anesthetic (100 mg/l) (MS-222, Sigma Chemical Co., St. Louis, Mo.) (See Appendix II, for details). The Stage I level of anesthesia (Houston et al., 1971), at which the fish lose equilibrium, was reached within one minute. Fish were kept in the anesthesia until the first flaring action of the operculum was observed (no longer than 4-5 minutes). They were then removed and placed ventral side up in an operating apparatus, consisting of a restraining cradle equipped with constant water irrigation of the gills (Appendix Figure 1).

A catheter (PE-50, polyethylene), with a short length of 23 gauge needle (Appendix Figure 2), was implanted from the ventral surface in the caudal artery (Appendix Figure 2). The catheters were filled with sterile saline prior to implantation. When correctly placed, blood pressure forced the saline out of the tubing. A syringe, containing a heparin/saline solution (1:4, v:v), was then connected to the tubing, and with gentle pressure the catheter was filled with enough solution to clear the needle portion of the catheter. The free end of the catheter was then sealed. The total operation took no more than 4-6 minutes to complete.

Fish were then replaced in separate compartments in the holding tanks. It was necessary to "swim" the animals until regular ventilation was resumed, and equilibrium regained. Prior to further use, recovery periods of 24 hours were provided. This is sufficient for return of Na^+, Cl^- and water distribution to the preoperative state (Houston et al., 1971).

Following this, 25 µl of [^{14}C]-polyethylene glycol-4000 (250 µCi/ml) was injected (Schmidt-Nielsen and Renfro, 1975). Subsequent to [^{14}C]-PEG administration, catheters were resealed, and a period of 12 hours provided to allow distribution of [^{14}C]-PEG. Extension of distribution times leads to PEG accumulation in the liver (Schmidt-Nielsen, 1977, personal communication).

Following equilibration, blood samples were withdrawn via the catheter, and fish sacrificed by severing the spinal cord. Tissue samples were taken as previously described. These were divided so that both ion analyses and radioactive counting could be performed. Prior to use, samples were held in sealed glass vials to reduce self-contamination.

Plasma and tissue water content, and electrolyte determinations were carried out as outlined earlier.

(b) Sample preparation for liquid scintillation counting

A Delta-3000 Liquid Scintillation Counter was used to determine [^{14}C]-PEG-4000 distribution (i.e., the amount of radioactivity) in plasma and tissue samples. These were prepared in the following fashion (See Appendix III for details). Small pieces of tissue ($\leq$ 0.2 g) were placed in pre-weighed glass scintillation vials, and their weights determined. 1.5 ml of NCS tissue solubilizer (Amersham/Searle Corp. Arlington Heights, Ill.) was added to each vial and samples heated at 50°C until totally solubilized

(3-4 hours). Because of the dark pigmentation of some tissues, it was necessary to add decolorizer. Vials were therefore cooled, and 0.2 ml of decolorizer solution (1.0 g benzoyl peroxide in 5.0 ml of toluene) added to each. The vials were then heated at 50°C for 30 minutes. After cooling 15.0 ml of a 2:1 ACS liquid scintillant:xylene mixture (Amersham/ Searle Corp.) were added to each vial. In the case of plasma, 100 µl samples were used, and these were treated in the manner outlined for tissues.

Since count rates (cpm) represent only a fraction of the actual disintegrations per minute (dpm) it is necessary to determine the counting efficiency, E; where E = cpm/dpm. Efficiency depends, largely, on the degree of quenching in the sample (Atallah et al., 1977). It was assumed that the greatest degree of quenching would be due to colour (Herzberg, 1960). A series of standards were therefore prepared. Each contained 10 µl of a 5 µl PEG-full strength per 1.0 ml of saline solution, whose theoretical dpm was 27,610.14 dpm $vial^{-1}$. $((1.2437 \times 10^{-3}$ µCi/µl) x $(2.22 \times 10^{-6}$ dpm) = 2,761.0 dpm/µl). To each of 6 vials, varying amounts (from 0-50 µl) of a stabilized hemoglobin solution (Hemotrol, Clinton Laboratories) were added and volume equalized at 50 µl with 0.7% saline. Thus, the vial containing no Hematrol contained 50 µl of saline, and was considered to be a blank with no color quenching. Blanks with no added radioactivity were also prepared. Standard and blank vials were treated in the same manner as sample vials with regard to addition of NCS solubilizer, decolorizer and scintillant (see Appendix III).

ESR, external standards ratio, appears to be the most widely used method of quench correction (Atallah et al., 1977). By comparing actual cpm and theoretical dpm of the standards and blanks with the external standards ratio, a quench curve was then constructed (ESR vs % Efficiency).

This allowed for estimation of counting efficiency for unknown quenched samples, once the ESR has been established.

The corrected cpm values were then used in the calculation of extracellular space as outlined by Schmidt-Nielsen *et al.* (1972).

$$ECS = \frac{T\ cpm}{(P\ cpm\ ml^{-1})(T\ wt)}$$

where: T cpm = total tissue cpm

P cpm ml^{-1} = cpm per ml of plasma

T wt = tissue wet weight, kg

ECS = extracellular space, ml/kg

5. Calculations:

Estimations of extracellular phase volumes on the basis of intrinsic ions were carried out using the following formulae:

(1) Chloride space

$$H_2O^{ecs}_{Cl}\ (ml/kg) = \frac{t\ Cl\ (0.9)}{p\ Cl} \times 1000$$ (Manery, 1954)

(2) Sodium space

$$H_2O^{ecs}_{Na}\ (ml/kg) = \frac{(t\ Na)(p\ H_2O)}{(p\ Na)(r\ Na)} \times 1000$$ (Manery, 1954)

(3) Chloride/potassium space

$$H_2O^{ecs}_{Cl/K}\ (ml/kg) = \frac{(t\ Cl)(i\ K) - (t\ H_2O)^2(i\ Cl)(i\ K)}{(t\ Cl)(i\ K) + (i\ Cl)(t\ K) - 2(t\ H_2O)(i\ Cl)(i\ K)} \times 1000$$

(Conway, 1957)

where t Cl = Tissue Cl^- concentration mM/kg

t Na = tissue Na^+ concentration, mM/kg

t K = tissue K^+ concentration, mM/kg

p Cl = plasma Cl^- concentration, mM/l

p Na = plasma Na^+ concentration, mM/l

p H_2O = plasma water content, ml/l

t H_2O = tissue water content, kg/kg

i Cl = interstitial fluid Cl^-,(mM/l); (p Cl·r Cl)

i K = interstitial fluid K^+,(mM/l); (p K·r K)

The Cl/K space formula found in Conway (1957) contains an error; the corrected version used here is taken from Hickman et al. (1964). r K^+, r Na^+ and r Cl^- refer to the Gibbs-Donnan ratio for the distribution of these ions between serum water and extracellular fluid as described in Manery (1954).

Cellular phase volumes were then calculated by subtracting extracellular phase volume from total tissue water, i.e.,

$$t\ H_2O - H_2O^{ecs} = H_2O^{ics}$$

Finally, cellular ionic concentrations were estimated using the relationship:

$$c\ X - \frac{t\ X - (p\ X)(H_2O^{ecs})}{H_2O^{ics}} \qquad \text{(Gordon, 1959)}$$

where c X = cellular concentration of electrolyte X, mM/l cell H_2O

t X = tissue concentration of X, mM/kg

p X = plasma concentration of X, mM/l

H_2O^{ecs} = extracellular phase volume, l/kg

H_2O^{ics} = cellular phase volume, l/kg

Equilibrium potentials for each electrolyte were also calculated using the Nernst relationship.

$$E_{ion} = \frac{R\ T}{F\ Z} \ln \frac{[ion]_o}{[ion]_i} v$$

where R = gas constant, 8.31 joules/mole-deg

T = absolute temperature

F = Faraday constant, 96,500 C/mole

Z = valency

6. Statistical Analysis:

All statistical analyses were carried out on a Wang 2200 desk top computer equipped with a line-printer. Specially designed programs (D. LeBlanc) allowed for the storing of data on tape cassettes and the subsequent automatic or manual handling of statistical analyses on the stored data.

Descriptive statistics--mean, variance, standard deviation, standard error and 95% confidence interval of the mean were first calculated. Single category analysis of variance were than carried out on base-10 logarithmically-transformed data. Differences at the 0.05 level or better were considered significant.

Correlation analyses were also carried out on all possible pairwise combinations of 84 variables/temperature group (included basic electrolyte concentrations and equilibrium potentials). The results of these are included only where relevant.

Raw data are recorded in appendix Tables 1 to 6 . The data are reported, in many cases, in more significant digits than necessary (as determined from rules governing use of significant digits) in the

appendix Tables. In most instances, the corresponding values reported in summary tables have been rounded off to the correct number of significant digits.

NOTE:

The word 'season' has been used throughout the results and discussion section to indicate those changes that are not a result of temperature alteration, but due apparently to the differing times of year in which sampling was carried out.

IV Results and Discussion

As noted in the Introduction, this investigation had three principal objectives. The first of these lay in comparison of extracellular phase volumes as defined by the widely-accepted use of [^{14}C]-PEG-4000, and older, presently less well-accepted procedures involving the distribution of intrinsic ions. It was hoped that one or more of the latter would provide estimates consistent with PEG values, and permit elimination of a time-consuming feature of the study. In addition, the use of these spaces in determining cellular electrolyte concentrations was evaluated.

The second aim was that of expanding the information available with regard to water-electrolyte distribution and thermal acclimation. As pointed out earlier, most studies in this area have considered only one or two tissues (usually plasma and muscle), and, ordinarily, only tissue electrolyte concentrations. The present study considers skeletal muscle, including possible site variations along the main epaxial muscle band, cardiac muscle, liver, spleen, gut tract and brain. Plasma and tissue, Na^+, K^+ and Cl^-, water content and distribution, and cell ionic concentrations were estimated on the basis of the extracellular spaces determined at three different acclimation temperatures.

Finally, since it is known that seasonally-appropriate variations in photoperiod (i.e., a long photoperiod corresponds to a summer situation) combine with temperature to alter water-electrolyte balance, seasonal differences in ionic regulation were considered.

The results obtained in this study are considered under two major headings:

(1) Space evaluation study, and

(2) Thermal acclimation study.

1. Space Evaluation Study

Raw data for the space evaluation study are presented in Appendix IV, Tables 1a to 1i.

A. Water-electrolyte levels

Table 3 summarizes water and electrolyte content data for plasma, and the eight tissue samples considered. The values obtained compare favorably with those previously reported for rainbow trout (Hickman et al., 1964; Houston et al., 1968; Murphy and Houston, 1977).

B. Extracellular phase volume and cellular ion concentration

As noted earlier, [^{14}C]-polyethylene glycol-4000 (PEG-4000) was used as an extrinsic extracellular marker, and values for extracellular space ($H_2O^{ecs}_{PEG}$) obtained in this way were compared with those resulting from ion-defined spaces ($H_2O^{ecs}_{Cl}$, $H_2O^{ecs}_{Cl/K}$, $H_2O^{ecs}_{Na}$).

Extracellular space values are summarized in Table 4, and graphically presented in Figure 3. The various tissues will be dealt with separately.

(i) Epaxial muscle

Three muscle sites were sampled--post-opercular, mid-dorsal and caudal muscle. The corresponding extracellular phase volumes, as estimated by the various techniques, are indicated in Figure 3a, b and c.

It will be apparent that PEG and Cl^- spaces did not differ significantly, and both were greater than the Cl/K space in all skeletal muscle samples. However, this was significant ($P < 0.05$) only in mid-dorsal muscle samples, where differences ranging from 7-12 ml kg^{-1} were encountered. Na^+ spaces

TABLE 3. Sodium, potassium and chloride levels (mM/l or mM/kg) and water content (g/kg) in plasma and tissues of rainbow trout. Reported as mean ± 1 standard error of the mean (N=33).

TISSUE	Na^+	Cl^-	K^+	H_2O
Plasma	134.34 ±3.23	128.24 ±2.80	3.47 ±0.16	966.3 ±2.05
Postopercular Muscle	11.76 ±0.54	5.99 ±0.15	149.94 ±2.24	763.6 ±1.79
Middorsal Muscle	10.41 ±0.48	5.24 ±0.16	148.78 ±1.58	761.8 ±2.21
Caudal Muscle	15.76 ±0.81	9.80 ±0.52	139.63 ±2.66	776.9 ±2.34
Cardiac Muscle	39.52 ±1.65	28.82 ±0.68	79.23 ±3.11	804.5 ±1.62
Liver	34.61 ±1.31	41.05 ±1.08	113.20 ±2.97	755.4 ±3.13
Spleen	35.62 ±2.37	43.05 ±1.37	103.74 ±2.77	775.4 ±4.45
Gut	66.28 ±1.90	61.30 ±1.61	65.65 ±2.85	804.0 ±2.00
Brain	69.29 ±1.75	44.45 ±1.19	77.30 ±3.17	803.0 ±2.00

TABLE 4. Extracellular phase volume estimates (ml/kg) using ^{14}C-PEG-4000 space, Cl^- space, Cl/K space and Na space. Reported as mean ± 1 standard error of the mean.

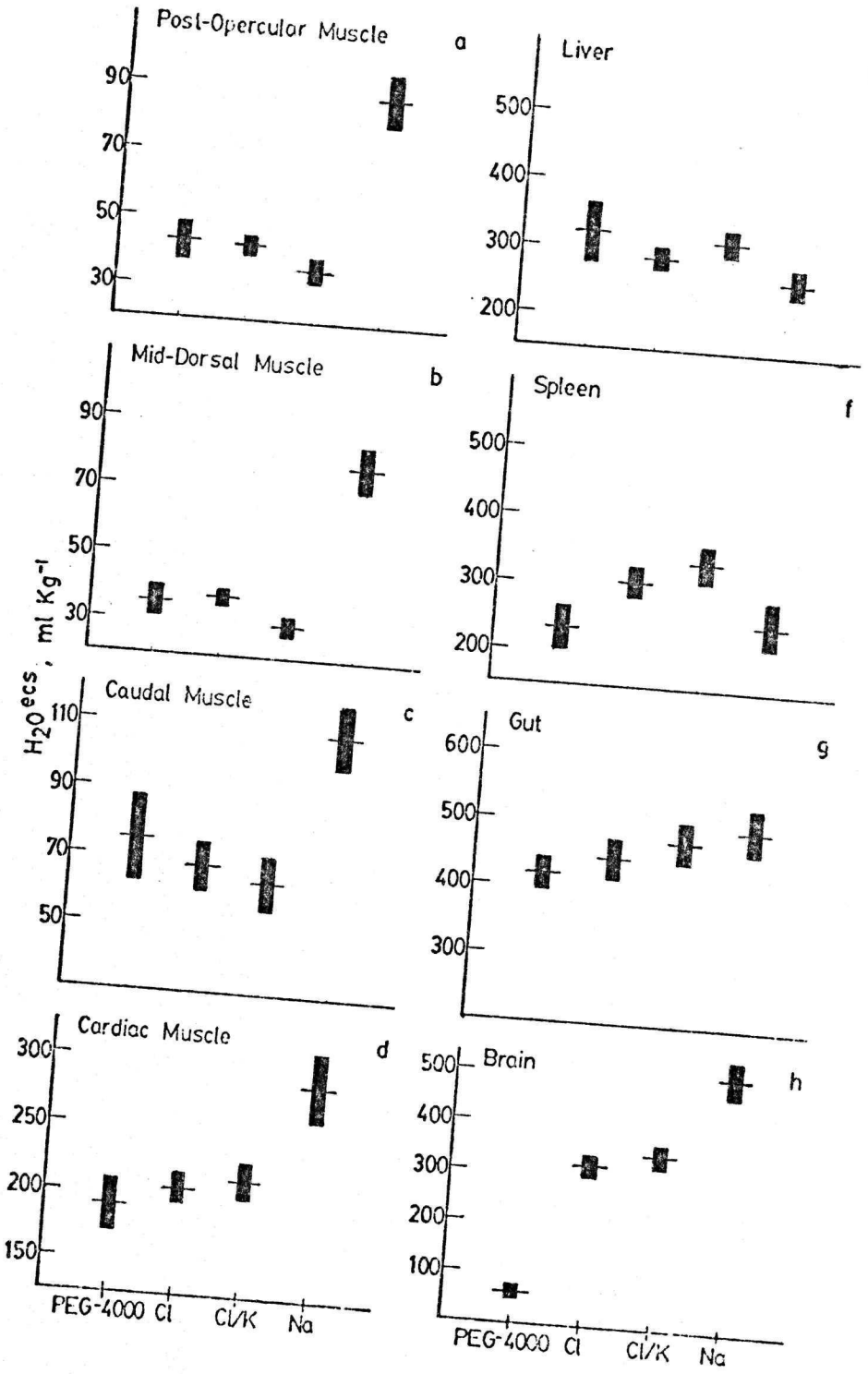
TISSUE	$H_2O^{ecs}_{PEG}$	$H_2O^{ecs}_{Cl}$	$H_2O^{ecs}_{Cl/K}$	$H_2O^{ecs}_{Na}$
Postopercular Muscle	43.03 ±2.66	42.48 ±1.26	35.93 ±1.74	87.37 ±3.81
Middorsal Muscle	35.96 ±2.13	37.14 ±1.14	29.53 ±1.58	77.36 ±3.37
Caudal Muscle	75.15 ±6.22	67.41 ±3.43	63.09 ±3.62	107.80 ±4.65
Cardiac Muscle	190.30 ±9.21	204.20 ±5.32	212.70 ±6.77	284.20 ±12.57
Liver	321.80 ±21.03	287.20 ±8.00	313.90 ±9.49	259.50 ±10.85
Spleen	234.40 ±15.85	305.90 ±10.90	336.40 ±13.26	248.60 ±15.63
Gut	419.90 ±11.66	445.00 ±14.49	472.10 ±14.77	493.60 ±16.42
Brain	63.31 ±6.52	316.90 ±9.92	344.60 ±12.17	503.30 ±15.29

Figure 3 PEG-4000, Cl^-, Cl/K and Na^+ spaces (ml kg^{-1}) in tissues of rainbow trout.

Horizontal line--mean

Vertical bar--95% confidence interval

Fig. 3


Post-Opercular Muscle
a
Liver
e
Mid-Dorsal Muscle
b
Spleen
f
Caudal Muscle
c
Gut
g
Cardiac Muscle
d
Brain
h
H_2O^{ecs}, ml Kg^{-1}
PEG-4000
Cl
Cl/K
Na

at all sites were much larger ($P < 0.01$) than other estimates of extracellular phase volume. The differences between Na^+ spaces and the other estimates ranged from 33 to 51 ml kg^{-1}. It should be noted, also, that significant variations in extracellular phase volume occurred along the epaxial muscle band. The smallest values for ECPV were encountered in the mid-dorsal samples, while the caudal muscle samples had the largest ECPV. These variations were well correlated with regional differences in tissue sodium, potassium and chloride content (Table 3).

Cellular Na^+, K^+ and Cl^- concentrations estimated on the basis of each of the spaces are summarized in Table 5 and Figure 4a to 1.

Values for cell K^+ (the most abundant cellular electrolyte) based on PEG, Cl^- and Cl/K spaces were in good agreement (<1% difference). Use of the Na^+ space lead to significantly ($P < 0.01$) higher (13-15 mM l^{-1}) values.

Cell Cl^- estimates based on the PEG (i.e., for post-opercular muscle, 0.64 ± 0.38 mM l^{-1} cell H_2O) and Cl^- (0.83 ± 0.01 mM l^{-1} cell H_2O) spaces were similar, while those calculated with Cl/K spaces were significantly ($P < 0.01$) higher (i.e., 1.99 ± 0.13 mM l^{-1} cell H_2O). Use of Na^+ space leads to negative estimates of cell Cl^-.

Cell Na^+ concentrations calculated from PEG, Cl^- and Cl/K spaces were comparable, ranging from 7.43 ± 0.67 to 10.59 ± 1.07 mM l^{-1} cell H_2O, in the three muscle sites sampled. Not unexpectedly, the use of Na^+ space for this purpose resulted in unrealistic values for cellular Na^+ concentrations, ranging from 0.09 ± 0.12 to 0.45 ± 0.32 mM l^{-1} cell H_2O.

The overall pattern observed in the comparison of cellular electrolyte estimates based on each of the spaces was similar at the three muscle sites sampled. There were, however, variations in cell electrolyte content along

TABLE 5. Cellular electrolyte levels (mM/litre cell water) estimated using Cl, Cl/K, Na and PEG spaces in tissues of rainbow trout, Salmo gairdneri, acclimated to 10 C. Reported as mean ± 1 standard error of the mean (N=33).

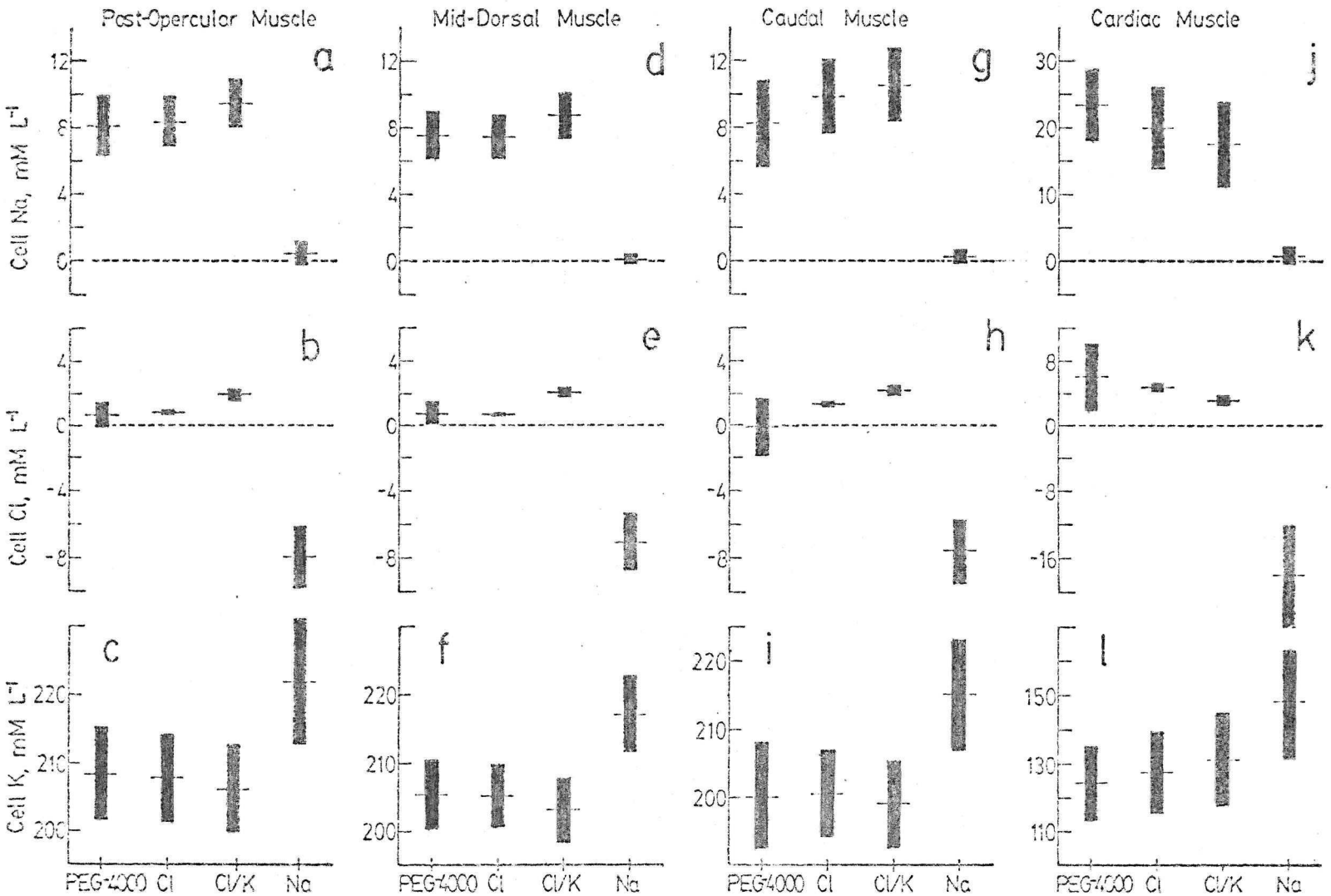
	Postopercular muscle	Middorsal muscle	Caudal muscle	Cardiac muscle	Liver	Spleen	Gut	Brain
Na estimated with Cl space	8.38 ±0.73	7.43 ±0.67	9.87 ±1.08	19.99 ±2.98	-11.31 ±4.48	-12.84 ±6.03	22.99 ±5.32	52.39 ±5.05
Cl/K space	9.49 ±0.72	8.75 ±0.68	10.59 ±1.07	17.54 ±3.14	-21.13 ±5.51	-24.26 ±7.27	5.94 ±7.01	46.00 ±6.04
Na space	0.45 ±0.32	0.09 ±0.12	0.26 ±0.18	0.80 ±0.62	0.37 ±0.63	0.41 ±0.73	1.47 ±2.12	3.00 ±2.26
PEG space	8.15 ±0.88	7.57 ±0.69	8.29 ±1.31	23.49 ±2.61	-31.84 ±10.25	0.14 ±7.05	24.70 ±5.34	82.17 ±2.54
K estimated with Cl space	207.86 ±3.19	205.22 ±2.28	200.69 ±3.16	127.70 ±5.98	239.65 ±8.16	215.76 ±10.11	180.87 ±11.68	160.28 ±7.88
Cl/K space	206.10 ±3.26	203.18 ±2.31	199.04 ±3.17	131.39 ±6.68	253.59 ±9.69	236.55 ±9.72	204.39 ±13.71	171.56 ±9.65
Na space	221.99 ±4.55	217.32 ±2.69	215.07 ±3.91	148.23 ±7.68	230.05 ±8.85	199.29 ±6.87	210.51 ±13.79	264.65 ±16.11
PEG space	208.39 ±3.32	205.43 ±2.54	200.38 ±3.84	124.56 ±5.24	283.07 ±19.19	200.29 ±9.98	167.55 ±8.34	104.15 ±4.03
Cl estimated with Cl space	0.83 ±0.02	0.72 ±0.02	1.37 ±0.07	4.83 ±0.14	8.74 ±0.29	9.40 ±0.46	17.55 ±0.77	9.32 ±0.42
Cl/K space	1.99 ±0.13	2.06 ±0.13	2.18 ±0.13	3.11 ±0.32	1.46 ±0.38	1.11 ±0.37	0.82 ±0.68	1.91 ±0.44
Na space	-7.95 ±0.93	-7.02 ±0.83	-7.58 ±0.91	-18.06 ±3.90	13.93 ±3.47	15.08 ±4.08	-4.52 ±4.81	-49.22 ±6.39
PEG space	0.64 ±0.38	0.79 ±0.34	-0.09 ±0.88	6.03 ±2.05	-12.03 ±8.49	17.86 ±3.70	13.31 ±5.40	49.16 ±1.52

Figure 4 Cell levels of sodium, potassium and chloride (mM l^{-1} cell water) in epaxial and cardiac muscle as calculated using PEG-4000, Cl^-, Cl/K and Na^+ space estimates

Horizontal line--mean

Vertical bar--95% confidence interval

Fig. 4


Post-Opercular Muscle
Mid-Dorsal Muscle
Caudal Muscle
Cardiac Muscle
a
b
c
d
e
f
g
h
i
j
k
l
Cell Na, mM L⁻¹
Cell Cl, mM L⁻¹
Cell K, mM L⁻¹
PEG-4000
Cl
Cl/K
Na

the epaxial muscle band. For example, caudal muscle was characterized by larger amounts of the predominantly extracellular electrolytes, Na^+ and Cl^-, and lesser amounts of K^+, the major cellular electrolyte, than either post-opercular or mid-dorsal muscle samples.

It is important to note that even large discrepancies in ECPV estimates do not lead to large differences in calculated cellular concentrations of predominantly cellular ions, such as K^+. On the other hand, small variations in ECPV will cause large discrepancies in the calculated cellular concentrations of electrolytes largely confined to the extracellular compartment, Na^+ and Cl^- (Sutton, 1968). This is apparent in Table 5 and Figure 4a to i.

(ii) Cardiac Muscle

Extracellular phase volume estimates for cardiac muscle based on each of the four spaces are shown in Figure 3d and Table 4. Estimates of cardiac muscle extracellular phase volume were much larger than that of skeletal muscle, and presumably reflect the larger amounts of Na^+ and Cl^- present in heart muscle. However, the relationship between the PEG, Cl^- and Cl/K spaces was similar to that seen in skeletal muscle, i.e., $H_2O^{ecs}_{PEG}$ = 190.3 ± 9.2 ml kg^{-2}; $H_2O^{ecs}_{Cl}$ = 204.2 ± 5.3 ml kg^{-1}; $H_2O^{ecs}_{Cl/K}$ = 212.7 ± 6.8 ml kg^{-1}. $H_2O^{ecs}_{Na}$ was again significantly ($P < 0.01$) higher (284.2 ± 12.6 ml kg^{-1}).

Cell K^+ levels (Fig. 4-1) calculated from the PEG space (124.6 ± 5.24 mM l^{-1} cell H_2O), Cl^- space (127.7 ± 5.98 mM l^{-1} cell H_2O) and Cl/K space (131.3 ± 6.68 mM l^{-1} cell H_2O) were in good agreement, while Na^+ space lead to much higher ($P < 0.05$) estimates (148.2 ± 7.68 mM l^{-1} cell H_2O).

Cellular Na^+ concentrations based on PEG (23.49 ± 2.61 mM l^{-1} cell H_2O), Cl^- (19.99 ± 2.98 mM l^{-1} cell H_2O) and Cl/K (17.54 ± 3.14 mM l^{-1} cell H_2O)

spaces were also comparable, whereas use of Na^+ of space lead to much lower values (0.80 ± 0.62 mM l^{-1} cell H_2O).

Cell Cl^- levels as estimated on the basis of PEG space were more variable (6.03 ± 2.05 mM l^{-1} cell H_2O), but again, there were no significant differences between this, and estimates based on the Cl^- (4.83 ± 0.14 mM l^{-1} cell H_2O) and Cl/K spaces (3.11 ± 0.32 mM l^{-1} cell H_2O). Use of the Na^+ space produced highly variable and negative values.

By comparison with skeletal muscle cellular electrolytes, cardiac muscle cell Na^+ and Cl^- concentrations were much higher and cell K^+ was much lower.

(iii) Liver:

Liver differed from all other tissues. The PEG-4000 space (321.7 ± 21.0 ml kg^{-1}) was larger, and the Na^+ space (259.5 ± 10.85 ml kg^{-1}) smaller than the $Cl^{-1}$and Cl/K spaces (Fig. 3c and Table 4). However, because of the relatively large variability of estimated PEG spaces, no significant differences were seen between this, and the Cl^- or Cl/K spaces. Significant ($P < 0.05$) variations existed between the three ion-defined spaces, and between PEG and Na^+ spaces ($P < 0.01$).

Cell K^+ estimates based on use of the three ion defined spaces (Fig. 5c, Table 5) gave comparable results: K^+_{Cl} = 239.6 ± 8.16; $K^+_{Cl/K}$ = 253.6 ± 9.69; K^+_{Na} = 230.1 ± 8.85 mM l^{-1} cell H_2O), while use of the PEG space yielded larger, more variable estimates (K^+_{PEG} = 283.1 ± 19.19 mM l^{-1}).

Cell Cl^- concentrations estimated from PEG (-12.03 ± 8.49 mM l^{-1} cell H_2O) and Na^+ spaces (13.93 ± 3.47 mM l^{-1} cell H_2O) were quite variable, and represented the two extremes of Cl^- concentrations Those based on Cl^- (8.74 ± 0.29 mM l^{-1}) and Cl/K spaces (1.46 ± 0.38 mM l^{-1}) were less variable

Figure 5 Cell levels of sodium, chloride and potassium (mM l^{-1} cell water) in liver, spleen, gut and brain as calculated using PEG-4000, Cl^-, Cl/K and Na^+ space estimates.

Horizontal line--mean.

Vertical bar--95% confidence interval.

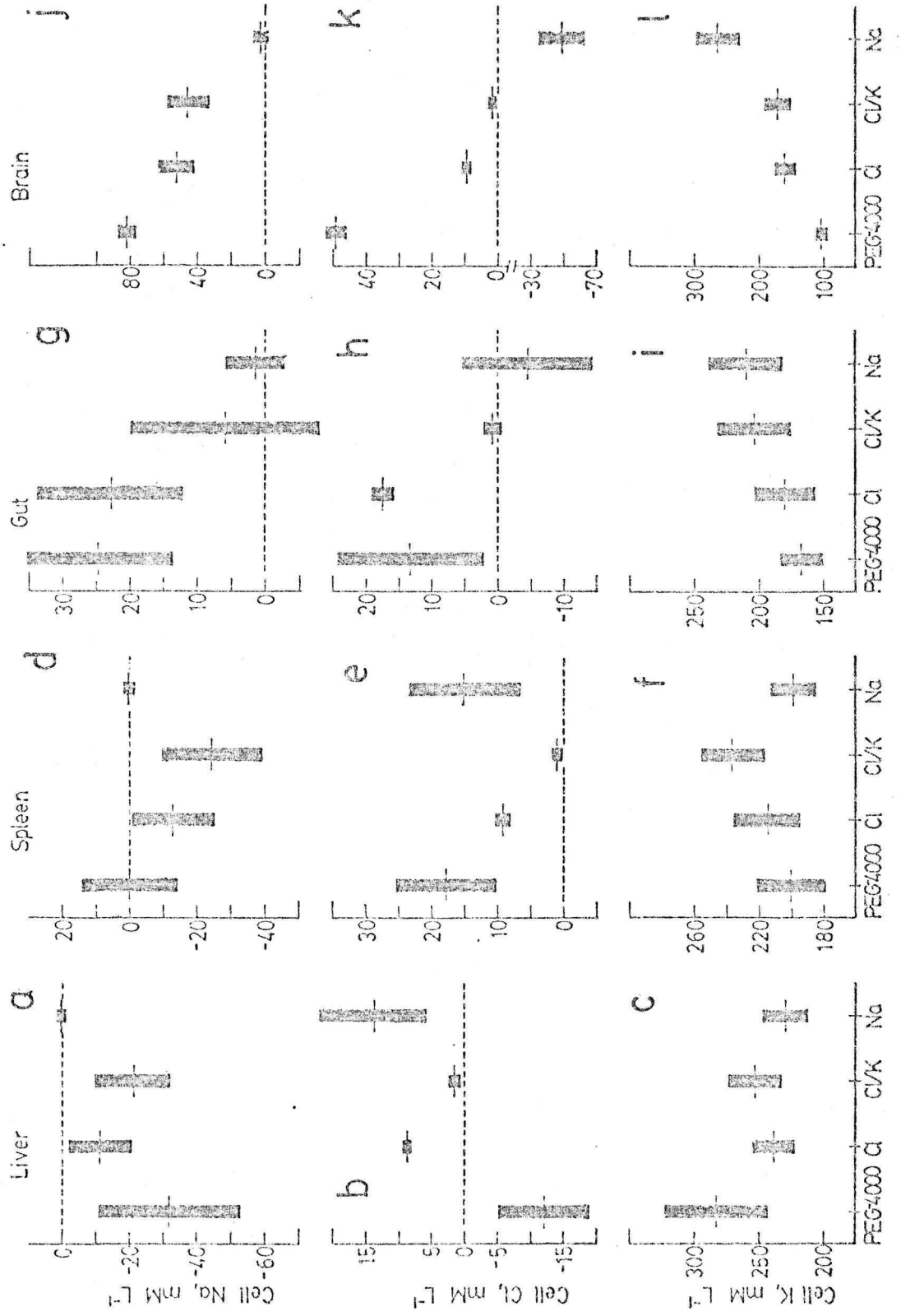

a
Liver
Cell Na, mM L⁻¹
0
-20
-40
-60
b
Cell Cl, mM L⁻¹
15
5
0
-5
-15
c
Cell K, mM L⁻¹
300
250
200
PEG4000 Cl Cl/K Na
d
Spleen
20
0
-20
-40
e
30
20
10
0
f
260
220
180
PEG4000 Cl Cl/K Na
g
Gut
30
20
10
0
h
20
10
0
-10
i
250
200
150
PEG4000 Cl Cl/K Na
j
Brain
80
40
0
k
40
20
0
-30
-70
l
300
200
100
PEG4000 Cl Cl/K Na

Fig. 5

provided values intermediate to those obtained from the former spaces (Fig. 5b, Table 5). All spaces produced negative estimates for cellular Na^+ in the liver (Fig. 5a, Table 5).

(iv) Spleen:

Extracellular phase volume estimates for spleen are presented in Figure 3f and Table 4. In spleen, unlike any other tissue, PEG (234.4 ± 15.9 ml kg^{-1}) and Na^+ spaces (248.6 ± 15.6 ml kg^{-1}) were similar. Cl^- (305.9 ± 10.9 ml kg^{-1}) and Cl/K space (336.4 ± 13.3 ml kg^{-1}) were comparable, and both were significantly ($P < 0.01$) larger than the former.

Cellular electrolyte estimates are displayed in Fig. 5 d to f.

Cell K^+ concentrations determined by means of $H_2O^{ecs}_{PEG}$ and $H_2O^{ecs}_{Na}$ were similar, while those based on $H_2O^{ecs}_{Cl}$ and $H_2O^{ecs}_{Cl/K}$ were higher, although only the use of $H_2O^{ecs}_{Cl/K}$ lead to significant ($P < 0.05$) differences (Fig. 5 f).

Cell Cl^- levels based on uses of PEG (17.86 ± 3.70 mM l^{-1} cell H_2O) and Na spaces (15.08 ± 4.08 mM l^{-1} cell H_2O) also gave comparable results. Use of Cl^- and Cl/K spaces yielded lower, less variable estimates (Fig. 5e).

As was the case with liver, estimates of cell Na^+, regardless of the space used, were negative or not significantly different from zero (Fig. 5d).

(v) Gut:

Extracellular phase volume estimates for gut tissue were much larger than those of the other tissues except brain ($H_2O^{ecs}_{Na}$). This may be related to the fact that gut tissues have unusually high levels of the predominantly extracellular ions, Na (66.28 ± 1.90 mM kg^{-1}) and Cl (61.30 ± 1.61 mM kg^{-1}). PEG space (419.90 ± 11.66 ml kg^{-1}) was similar only to the Cl^- space (445.0 ± 14.49 ml kg^{-1}). Both Cl/K (472.10 ± 14.77 ml kg^{-1}) and Na^+ spaces (493.60 ± 16.42 ml kg^{-1}) differed significantly ($P < 0.05$) from PEG

space (Fig. 3g, Table 4), but were not themselves significantly different.

Cellular K^+ estimates (Fig. 5i) based on Cl^- (180.8 ± 11.80 mM l^{-1} cell H_2O) and PEG spaces (167.5 ± 8.30 mM l^{-1} cell H_2O) agreed closely, while those based on Cl/K (204.4 ± 13.7 mM l^{-1}) and Na^+ spaces (210.5 ± 13.8 mM l^{-1}) were higher.

In the case of chloride, PEG and Cl^- space-based estimates were, again, close, although Cl^-_{PEG} was more variable (13.31 ± 5.40 mM l^{-1}) than $Cl^-_{Cl^-}$ (17.55 ± 0.77 mM l^{-1}). Both the Cl/K and Na^+ spaces lead to smaller, and, in the case of the Na^+ space, negative values for cellular Cl^- (Fig. 5h, Table 5).

Estimates of cellular Na^+ were also variable, but again those based on use of PEG and Cl^- spaces were in closest agreement, while Cl/K and Na^+ species yielded much smaller volumes (Fig. 5g, Table 5).

(vi) Brain

The situation observed for brain was different from those seen in other tissues. The PEG space (63.31 ± 6.52 ml kg^{-1}) was much smaller ($P < 0.01$) than any of the ion-defined spaces (Fig. 3h). The Cl^- (316.9 ± 9.92 ml kg^{-1}) and Cl/K space (344.6 ± 12.2 ml kg^{-1}), although significantly different ($P < 0.05$), were closest in value, while Na^+ space yielded very large estimates of ECPV (503.3 ± 15.3 ml kg^{-1}). PEG space was similar to that of skeletal muscle, while the ion-defined spaces resembled those obtained for liver, spleen and gut.

Because of large differences in estimates of ECPV, large variations in estimated cellular electrolyte concentrations were encountered (Table 5, Fig. 5 j to 1).

Lowest values for cellular K^+ were obtained using PEG space (104.2 ± 4.08 mM l^{-1} cell H_2O), with Cl^- (160.3 ± 7.88 mM l^{-1}) and Cl/K

(171.6 ± 9.65 mM l^{-1}) spaces providing values of intermediate magnitude, and Na space (264.7 ± 16.1 mM l^{-1}) giving the highest values (Fig. 5-1).

This situation was reversed in the case of cell Cl^- (Fig. 5k). Use of $H_2O^{ecs}_{PEG}$ gave the highest estimates (49.16 ± 1.52 mM l^{-1}), $H_2O^{ecs}_{Na}$ yielded negative results, and $H_2O^{ecs}_{cl}$ and $H_2O^{ecs}_{Cl/K}$ estimates were of intermediate value.

This was also observed in the case of cell Na^+, with use of $H_2O^{ecs}_{PEG}$ giving the highest estimates and $H_2O^{ecs}_{Na}$ the lowest (Fig. 5-1). In addition, cellular Na^+ concentrations obtained using any of the spaces, with the exception of the Na^+ space, were higher than those seen in any of the other tissues.

(C) Discussion of Space Evaluation

The body fluid system can be divided into a number of phases, and within major phases, a number of subphases. It is a gross oversimplification to view the body fluid system in terms of only extra- and intracellular compartments. Manery (1954) for example, suggested that the extracellular compartment could be legitimately partitioned into

(i) a physiological extracellular phase, consisting of the plasma water, and extravascular fluids into which ions and small molecules can diffuse freely, i.e., fluids which are in more-or-less free communication; and

(ii) an anatomical extracellular phase, which is defined by discernible boundaries, and which includes secretions, cerebrospinal fluid and eye humours.

From the functional point of view, the critical portion of the extracellular compartment is the immediate cellular environment, which can be partitioned into interstitial and connective tissue subphases. No anatomical boundaries are seen here, although a functional subdivision is possible in terms of ease of solute diffusion. Because there are no definite boundaries

to the extravascular component of the extracellular phase, the values obtained for this phase must be regarded as estimates. However, such estimates are regarded as acceptable approximations of the physiologically functional extracellular phase volume (ECPV), because the rates of solute movement and distribution within this 'physiological' ECPV are much faster than those from this area into the other compartments of the body fluid system (Manery, 1954).

Various solutes have been used in the past to determine ECPV, including sodium, chloride, sucrose and polyethylene glycols of various molecular weights. Difficulties exist in the accurate assessment of ECPV with each of these. The use of chloride and sodium in the determination of $H_2O^{ecs}_{Cl}$ and $H_2O^{ecs}_{Na}$ (Manery, 1954) assumes that these ions are confined exclusively to the extracellular compartment. This is not so in any tissue (e.g., Gordon, 1959; Forbes, 1962; Cotlove and Hogben, 1962). The Cl/K space of Conway (1957) assumes a Donnan relationship with respect to the distribution of this ion pair between cellular and extracellular phases, a situation which is, in fact, demonstrable in the instance of skeletal muscle (Cotlove and Hogben, 1962).

Extrinsic substances such as inulin, polyethylene glycol and iothalamate, are regarded as providing more realistic estimates of the ECPV. There are problems with this approach as well. These solutes must be injected or administered via in situ catheters. Implantation procedures, however, stress the animal. In fishes, for example, handling, anesthetization and catheter insertion lead to alterations in tissue water content and distribution which persist for many hours (Houston et al., 1969, 1971). Return to steady levels resembling the pre-operative state require at least 24 hours. In any case values for ECPV obtained in this manner tend to agree rather

closely with those determined from intrinsic solutes.

[^{14}C]-polyethylene glycol-4000 (PEG-4000), which is an inert, uncharged molecule, large enough to be excluded from the cells, and small enough to distribute itself fairly quickly in the extracellular space (McIver and Macknight, 1974), seems to be the most reliable polymer for use in ECPV determinations in teleosts (Beyenbach and Kirschner, 1976). The latter authors found that other marker substances (commonly used in mammalian studies) such as inulin or glofil (iothalamate) were not effectively confined to the extracellular phase. Schmidt-Nielsen et al. (1972) concluded that [^{14}C]-PEG-4000 seemed to approximate the ECPV more closely than inulin (in eel, Anguilla rostrata, and flounder Pseudopleuronectes americanus), because it produces lower volumes-of-distribution, and gives more consistent estimates of cellular ion concentrations resembling those of other vertebrates, than was the case with inulin. Tritiated-labelled compounds were found to be unreliable, since they are either metabolized or accumulated by both liver and kidney (Schmidt-Nielsen et al., 1972). These authors also noted that [^{14}C]-PEG-4000 distribution should be measured within 12 to 24 hours of injection, since there is some evidence that it, too, is slowly accumulated by the liver (Schmidt-Nielsen, 1977, personal communication). Within the foregoing limitations, however, [^{14}C]-PEG-4000 appears to be the most reliable of the currently used extrinsic extracellular markers.

Values for $H_2O^{ecs}_{Cl}$, $H_2O^{ecs}_{Cl/K}$ and $H_2O^{ecs}_{Na}$ in skeletal muscle obtained in this study were in good agreement with those of previous studies involving rainbow trout (Hickman et al., 1964; Houston et al., 1968; Murphy and Houston, 1977). Those for liver were comparable with estimates made by Murphy and Houston (1977). Hickman et al. (1964) provide data on brain ECPV, and this, too, is in agreement with values obtained in this study.

Ward and Stokes (1969) reported values for gut extracellular space comparable to those found in the present study, although their determinations were done on gut segments incubated *in vitro* with (carboxy-^{14}C)-inulin. No other recent literature dealing with extracellular phase volumes in cardiac muscle, gut or spleen of rainbow trout, or any other fish, seems to be available.

In this investigation, values for muscle and liver $H_2O^{ecs}_{PEG}$ were comparable to those reported by Schmidt-Nielsen *et al.* (1972) for freshwater-adapted eel. Lutz (1972) obtained good agreement between Cl^- and unlabelled inulin spaces of perch, *Perca fluviatilis*, muscle, and reasonable agreement between the Na^+ and inulin spaces of perch liver. Cl^- spaces reported by Lutz (1972) for perch liver were, however, substantially higher. The Na space of rainbow trout liver encountered in this study corresponded with that of perch liver, and was also less than the Cl^- space, although the difference was not as large. However, the PEG space of trout liver was larger than the inulin space of perch liver.

If it is assumed that the PEG space is a good approximation of the actual extracellular phase volume, then it is possible to determine which of the ion-defined spaces should be used with each tissue to provide acceptable assessments of the ECPV. To ascertain this, correlation analyses were carried out to show the relationship between PEG space and each of the ion-defined values for ECPV (Table 6). Cellular electrolyte concentrations obtained using each space were also compared, and taken into consideration in order to determine which of the latter provided acceptable estimates of ECPV.

Since all three epaxial muscle sites gave essentially similar values, they were treated as a single group. Correlations between PEG and Cl^- space,

TABLE 6. Correlation coefficients for ^{14}C-PEG-4000 spaces compared with Cl^-, Cl/K and Na^+ spaces, with sample size (N) and level of significance.

TISSUE	$H_2O^{ecs}_{PEG}$ vs. $H_2O^{ecs}_{Cl}$	$H_2O^{ecs}_{PEG}$ vs. $H_2O^{ecs}_{Cl/K}$	$H_2O^{ecs}_{PEG}$ vs. $H_2O^{ecs}_{Na}$
Postopercular Muscle	0.535 (32) $P<0.01$	0.437 (32) $P<0.05$	0.117 (32) NS
Middorsal Muscle	0.547 (32) $P<0.01$	0.514 (32) $P<0.01$	0.153 (32) NS
Caudal Muscle	0.690 (29) $P<0.01$	0.691 (29) $P<0.01$	0.525 (29) $P<0.01$
Cardiac Muscle	0.169 (32) NS	0.110 (32) NS	0.345 (30) NS
Liver	0.098 (29) NS	0.069 (29) NS	0.282 (30) NS
Spleen	0.471 (29) $P<0.01$	0.307 (28) NS	0.702 (25) $P<0.01$
Gut	0.065 (28) NS	0.049 (28) NS	0.274 (28) NS
Brain	0.339 (32) NS	0.296 (32) NS	0.119 (31) NS

and PEG and Cl/K space were significant ($P < 0.05$). Na^+ spaces were not significantly correlated with PEG spaces in two of the three samples. Furthermore, Na^+ space was consistently much larger than the others, and cellular electrolyte concentrations estimated from use of the Na^+ space were not comparable to those based on use of PEG, Cl and Cl/K spaces. Of the ion-defined spaces, that based on Cl/K distribution consistently gave the lowest estimates of ECPV. This was, therefore, chosen as the ion-defined space best estimating ECPV. There several reasons underlying this choice. As mentioned earlier, this means of estimating ECPV is based on an assumed Donnan distribution of Cl^- and K^+ between the extracellular and intracellular fluid. Cotlove and Hogben (1962) have provided evidence which supports this assumption in the case of muscle. The second reason for selection of the Cl/K space lies in the fact that, by comparison with Cl^- and Na^+ spaces, it yields the lowest estimate of ECPV.

Review of the literature indicates that investigators have tended to choose procedures which yield the lowest estimate of ECPV, when dealing with ion-defined spaces (Schmidt-Nielsen, et al., 1972; Beyenbach and Kirschner, 1976). Implicit in this choice, although rarely if ever stated, is the fact that ion-defined spaces overestimate the true extracellular volume. Obviously, extrinsic solutes may also overestimate ECPV, if they diffuse beyond the extracellular compartment. Because of this "lowest values" for ECPV may be the best indicators of actual ECPV. The second criterion for procedure selection is the use of a technique which yields consistent values for cellular electrolyte concentrations, with low variability.

Accordingly, these criteria have been used in this investigation for selection of spaces which best approximate ECPV.

Cardiac muscle presents a somewhat different case. Heart muscle contains more Na^+, more Cl^- and less K^+ than skeletal muscle. This points to the likelihood of a larger extracellular volume, since these are predominantly extracellular ions. None of the correlations between PEG space and the ion-defined spaces proved to be significant. PEG and Cl and Cl/K spaces were however, comparable, while Na^+ space was much larger. The Cl^- space was chosen as the best approximation of ECPV on the basis of the criteria defined above. The Cl^- space, in comparison with the other ion-defined spaces, provided the lowest estimates of ECPV, and also gave the most consistent results for cellular electrolytes.

In gut tissue, the Cl^- space again was taken as providing the closest approximation of PEG space. Although none of the correlations proved significant, the Cl^- space seemed to provide more consistent estimates of cellular electrolyte levels and values similar to those determined using PEG space. The rather high concentration of intracellular Cl^- is thought to be due to the presence of glands in the mucosal layer of the gastrointestinal tract (Manery, 1954; Lutz, 1972). In addition, Cl^- can be absorbed by the intestine along with Na^+ (Conte, 1969). The apparent lack of cellular Na^+ is not readily explainable.

The situation with respect to brain was particularly difficult to assess because of the large discrepancies between PEG and other spaces. There are several possible reasons for this. The extracellular phase of brain may be composed of two components: a fast-equilibrating phase and a slow-equilibrating phase (Lutz, 1972). It is possible that PEG may have equilibrated through only the first of these, or may only be accessible to the first, while the ions may have access to both compartments. The latter would, of course, account for the difference between PEG and ion-defined spaces. The

possibility of a two-phase extracellular compartment in brain was also suggested by Manery (1954). Difficulties in evaluating ion and water levels may also result from the fact that the brain is a complex organ with regional variations in both form and function. The best correlation (Table 6) was obtained between PEG and Cl^- spaces. The Cl^- space also provided more consistent results in later experiments.

In spleen, the best approximation of the PEG space came from the sodium space, unlike the other tissues. The correlation between these two spaces was highly significant ($P < 0.01$), and the cellular estimates based on each of these were very close in value (Fig. 5d to 1). Both Cl and Cl/K spaces appeared to overestimate the ECPV. The Na^+ space also gave the lowest estimates of ECPV, in agreement with the criteria discussed earlier. However, the spaces obtained for this organ may or may not be truly indicative of the actual situation. The spleen is a glandular organ, i.e., it is made up of a collagenous framework filled in by reticular and lymphatic tissue, forming a somewhat spongy network. In addition, the spleen contains a relatively large concentration of blood. This type of organization may lead to problems in accurately assessing the extracellular phase volume. and therefore, it is relevant to note that the values obtained for both ECPV and cellular electrolyte levels should be regarded only as estimated means for the entire compartment.

The liver contrasted with the other tissues in that the PEG space was somewhat larger than the ion-defined spaces, which might have resulted from possible accumulation of PEG within the liver. The best correlation observed was between PEG and the Na space, although this was not significant (Table 6). The Na^+ space was chosen as most closely approximating the extracellular space, on the basis of the criterion that it gave the smallest estimate

of ECPV. Like the spleen, however, liver is not an ideal tissue for space determinations. Problems arise because of the multi-compartmental nature of the liver, the presence of large amounts of blood and of large numbers of macrophages leading to non-specific uptake of extrinsic markers (Williams and Woodbury, 1971). Therefore the values obtained for both ECPV and cellular electrolytes should, again as in the spleen, be regarded as means for the compartments.

In summary, then, [^{14}C]-PEG-4000 was determined to be useful in the assessment of the extracellular phase volume in all tissues tested, with the exception of liver.

The Cl/K space was determined to be a good approximation of ECPV in skeletal muscle. On the other hand, Cl^- spaces appear to be the best estimates of ECPV in cardiac muscle, gut tissue and brain.

D. Summary:

1. [^{14}C]-PEG-4000 was determined to be useful in the assessment of the extracellular phase volume in all tissues tested, with the exception of liver,

2. PEG space was compared with each of the ion-defined spaces to determine which of these could provide resasonably accurate assessments of the ECPV of each tissue tested, and thus provide an alternative to the use of an extrinsic solute.

3. In the case of epaxial muscle, the Cl/K space was determined to be a good approximation of the extracellular phase volume.

4. For cardiac muscle, gut tissue and brain, the Cl^- space appeared to provide the best estimates of extracellular phase volume.

5. The Na^+ space was taken as being a fairly realistic approximation of the extracellular phase volume in liver and spleen.

6. It was noted that any inaccuracies in ECPV, regardless of spacing technique will have little effect on the calculated cellular concentrations of the predominantly intracellular cation, K^+. However, small variations in ECPV will have a much greater effect on the calculated cellular concentrations of the major extracellular ions, Na^+ and Cl^-.

2. Thermal Acclimation Studies

The second section of this investigation consisted of thermal acclimation studies. As noted previously, this involved groups of 17 to 27 rainbow trout acclimated to 2°, 10° and 18°C. Because water-electrolyte balance is altered by photoperiod, probably through the influence of this variable upon endocrine systems implicated in ionic regulation (Murphy and Houston, 1977), summer and fall-winter samples were taken in order to examine the possible existence of intrinsic seasonal differences in response to temperature under circumstances of constant 'neutral' photoperiod (12 hours light:12 hours darkness).

The first batch of trout sampled during July and August, 1975, have been termed 'summer series' fish; the second set, sampled in November and December, 1975, are referred to as 'fall' or 'fall-winter' series fish.

Values for plasma and tissue electrolyte levels and water content are summarized in Tables 7 and 8 for 'summer' fish, and Tables 9 and 10 for 'fall' fish.

Extracellular phase volume estimates based on the three ion-defined spaces were calculated for each tissue, and cellular electrolyte levels based on each of these spaces determined for each tissue. This was done to insure that the relationships between the various spaces were comparable to those observed in the space evaluation study. However, only those estimates which were obtained using the ion-defined space appearing to give the most reliable approximation of ECPV in each tissue have actually been included.

The results obtained in this aspect of the study will be dealt with by tissue, and discussed at the end of each tissue section.

All raw data for the thermal acclimation study are presented in Appendix V, Tables 1 through 6.

TABLE 7. Electrolyte levels (mM/litre or mM/kg) in plasma and tissues of rainbow trout (summer series) acclimated to 2, 10 and 18 C. Reported as mean ± 1 standard error of the mean (2 C, N=17; 10 C, N=17; 18 C, N=18).

	SODIUM			CHLORIDE			POTASSIUM		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Plasma	159.89 ±2.31	156.22 ±2.27	156.80 ±2.50	129.44 ±2.02	127.64 ±2.29	129.49 ±0.91	1.98 ±0.29	1.90 ±0.29	2.13 ±0.17
	-- NS --	-- NS --		-- NS --	-- NS --		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Postopercular Muscle	10.33 ±0.53	13.14 ±0.56	12.47 ±0.67	6.45 ±0.13	7.67 ±0.19	7.47 ±0.16	116.42 ±3.56	123.90 ±2.85	126.55 ±4.24
	--p<.01--	-- NS --		--p<.01--	-- NS --		-- NS --	-- NS --	
	------- p<.05 -------			------- p<.05 -------			-------- NS --------		
Middorsal Muscle	9.95 ±0.80	11.47 ±0.50	10.68 ±0.65	5.72 ±0.24	6.36 ±0.18	6.56 ±0.19	117.74 ±3.63	125.91 ±1.67	125.52 ±3.68
	-- NS --	-- NS --		--p<.05--	-- NS --		--p<.05--	-- NS --	
	-------- NS --------			-------- p<.01 ------			-------- NS --------		
Caudal Muscle	11.92 ±0.74	13.87 ±0.84	14.34 ±0.94	9.04 ±0.44	8.77 ±0.52	9.12 ±0.25	118.86 ±3.66	117.01 ±2.76	127.05 ±4.47
	-- NS --	-- NS --		-- NS --	-- NS --		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Cardiac Muscle	58.32 ±2.75	55.49 ±3.41	54.36 ±3.88	44.07 ±1.19	41.67 ±0.77	46.81 ±1.21	60.54 ±4.17	61.71 ±3.93	68.28 ±5.65
	-- NS --	-- NS --		-- NS --	--p<.01--		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Liver	30.82 ±1.51	36.99 ±1.37	32.66 ±1.33	42.87 ±0.79	52.66 ±1.19	49.29 ±0.97	73.96 ±6.53	92.35 ±3.24	86.21 ±2.14
	--p<.01--	--p<.05--		--p<.01--	--p<.05--		--p<.05--	-- NS --	
	-------- NS --------			------- p<.01 ------			------- p<.05 ------		
Gut	39.37 ±2.89	43.97 ±3.14	42.33 ±2.99	34.51 ±1.52	39.71 ±2.03	38.92 ±1.75	53.16 ±5.01	60.13 ±4.79	56.96 ±4.49
	-- NS --	-- NS --		-- NS --	-- NS --		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Brain	70.82 ±3.65	77.88 ±2.10	74.79 ±2.01	43.74 ±1.55	44.46 ±1.25	48.88 ±1.06	69.38 ±5.57	65.45 ±3.07	86.83 ±5.11
	-- NS --	-- NS -		-- NS --	--p<.01-		-- NS --	--p<.01-	
	-------- NS -------			------- p<.01 ------			------- p<.01 ------		

TABLE 8. Water content (g/kg or ml/l), extracellular phase volume (ml/kg) and intracellular phase volume (ml/kg) of tissues of rainbow trout (summer series) acclimated to 2, 10 and 18°C. Reported as mean ± 1 standard error of the mean. Sample sizes are as reported in Table 7.

	H_2O			H_2O^{ecs}			H_2O^{ics}		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Plasma	944.4 ±5.01	953.1 ±4.98	939.9 ±5.73	---	----	----	---	----	----
	- NS -	- NS -							
	------ NS ------								
Post-Opercular Muscle	788.3 ±0.96	791.9 ±2.50	788.8 ±1.91	41.1 ±1.91	51.8 ±2.09	49.5 ±1.50	747.1 ±1.97	738.2 ±3.63	740.2 ±1.98
	- NS-	- NS -		-p<.01-	- NS -		-p<.05-	- NS -	
	----- NS ------			----- p<.01 ----			------ p<.05 ----		
Mid-Dorsal Muscle	784.3	787.9	787.3	35.1	42.7	40.9	748.4	742.6	745.9
	±0.88	±2.25	±1.84	±2.82	±1.77	±1.83	±2.54	±2.56	±2.20
	- NS -	- NS -		-p<.05-	- NS -		- NS -	- NS -	
	------ NS ------			----- p<.05 ----			------ NS ------		
Caudal Muscle	786.9	791.7	791.0	57.9	60.0	63.5	728.7	730.0	728.4
	±1.13	±2.09	±1.99	±3.68	±1.50	±2.56	±4.30	±2.59	±2.83
	-p<.05-	- NS -		- NS -	- NS -		- NS -	- NS -	
	------- NS ------			------ NS ------			------ NS ------		
Cardiac Muscle	814.9	811.0	816.6	300.5	294.	324.8	515.8	516.7	495.7
	±2.44	±2.88	±2.36	±8.88	±4.90	±9.00	±9.15	±5.45	±10.02
	- NS -	- NS -		- NS -	-p<.01-		- NS -	- NS -	
	------ NS ------			------ NS ------			------ NS ------		
Liver	753.1	757.7	755.2	196.7	248.2	208.4	555.6	512.6	546.5
	±1.64	±2.68	±2.51	±10.28	±8.83	±9 97	±10.16	±8.84	±9.70
	- NS -	- NS -		-p<.01-	-p<.01-		-p<.01-	-p<.05-	
	------ NS ------			------- NS ------			------- NS ------		
Gut	785.6	782.6	793.7	242.6	279.8	270.3	543.7	501.5	520.1
	±3.95	±4.75	±4.45	±10.17	±13.01	±12.92	±12.10	±12.59	±16.62
	- NS -	- NS -		- NS -	- NS -		-p<.05-	- NS -	
	------ NS ------			------ NS ------			------- NS ------		
Brain	808.5	804.2	809.2	300.9	316.2	340.5	506.7	488.0	474.5
	±2.71	±1.26	±1.67	±11.31	±6.79	±9.23	±9.19	±7.31	±7.77
	- NS -	-p<.05-		- NS -	-p<.05-		- NS -	- NS -	
	------ NS ------			---- p<.01 -----			---- p<.05 -----		

TABLE 9. Electrolyte levels (mM/litre or mM/kg) in plasma and tissues of rainbow trout (fall series) acclimated to 2, 10 and 18 C. Reported as mean ± 1 standard error of the mean (2 C, N=27; 10 C, N=21; 18 C, N=18).

	SODIUM			CHLORIDE			POTASSIUM		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Plasma	147.24 ±1.94	153.57 ±1.01	150.72 ±1.85	133.49 ±1.09	133.10 ±0.74	132.56 ±1.70	1.45 ±0.13	1.56 ±0.18	3.12 ±0.32
	--p<.01--	-- NS --		-- NS --	-- NS --		--- NS ---	--p<.01--	
	-------- NS --------			-------- NS --------			--------p<.01--------		
Postopercular Muscle	11.81 ±0.46	13.82 ±0.54	15.00 ±0.56	6.63 ±0.16	7.69 ±0.16	7.86 ±0.19	145.46 ±1.94	146.16 ±1.48	149.25 ±0.97
	--p<.01--	-- NS --		--p<.01--	-- NS --		---NS --	-- NS --	
	------- p<.01 ------			------- p<.01 -------			-------- NS --------		
Middorsal Muscle	10.19 ±0.42	11.76 ±0.31	13.57 ±0.52	5.62 ±0.10	6.50 ±0.15	6.48 ±0.12	148.52 ±1.76	152.65 ±1.35	156.19 ±2.19
	--p<.01--	--p<.01--		--p<.01--	-- NS --		-- NS --	-- NS --	
	------- p<.01 -------			------- p<.01 -------			------- p<.01 ------		
Caudal Muscle	11.33 ±0.34	13.43 ±0.36	14.14 ±0.42	6.95 ±0.11	7.29 ±0.10	7.76 ±0.17	150.84 ±2.46	152.21 ±1.93	156.21 ±2.66
	--p<.01--	-- NS --		--p<.05--	--p<.05--		-- NS --	-- NS --	
	------- p<.01 -------			------- p<.01 -------			-------- NS --------		
Cardiac Muscle	43.19 ±1.27	46.75 ±1.21	48.06 ±1.12	31.96 ±0.59	32.83 ±0.62	35.76 ±0.62	39.19 ±0.59	46.66 ±2.62	39.12 ±2.06
	-- NS --	-- NS --		-- NS --	--p<.01--		--p<.05--	--p<.05--	
	------- p<.01 -------			------- p<.01 -------			-------- NS --------		
Liver	29.32 ±0.65	35.25 ±0.89	35.10 ±0.90	38.06 ±0.68	44.58 ±0.71	48.73 ±1.18	115.93 ±2.29	120.65 ±1.65	115.68 ±3.13
	--p<.01--	-- NS --		--p<.01--	--p<.01--		-- NS --	-- NS --	
	------- p<.01 -------			------- p<.01 -------			-------- NS --------		
Spleen	19.53 ±0.63	20.14 ±0.65	23.11 ±0.91	34.92 ±0.63	41.76 ±0.90	47.54 ±0.82	97.21 ±3.09	100.15 ±3.41	94.24 ±3.70
	-- NS --	--p<.05--		--p<.01--	--p<.01--		-- NS --	-- NS --	
	------- p<.01 -------			------- p<.01 -------			-------- NS --------		
Gut	54.91 ±1.37	59.21 ±2.27	59.26 ±1.90	55.28 ±1.19	61.27 ±2.13	59.44 ±1.88	45.45 ±2.66	50.87 ±3.27	41.31 ±2.74
	-- NS --	-- NS --		--p<.05--	-- NS --		-- NS --	--p<.05--	
	-------- NS --------			-------- NS --------			-------- NS --------		
Brain	64.32 ±2.17	74.11 ±2.08	73.01 ±1.81	42.54 ±1.25	50.52 ±1.31	48.27 ±1.29	33.01 ±1.52	37.03 ±2.08	34.52 ±2.15
	--p<.01--	-- NS --		--p<.01--	-- NS --		--p<.05--	-- NS --	
	------- p<.01 ------			------- p<.01 ------			------- NS ------		

TABLE 10. Water content (g/kg or ml/l), extracellular phase volume (ml/kg) and intracellular phase volume (ml/kg) of tissues of rainbow trout (fall series) acclimated to 2, 10 and 18°C. Reprrted as mean ± 1 standard error of the mean. Sample sizes are as reported in Table 9.

	H_2O			H_2O^{ecs}			H_2O^{ics}		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Plasma	937.0 ±2.78	937.9 ±2.43	936.8 ±2.92	---	----	----	---	----	----
	- NS -	- NS							
	------ NS ------								
Post-Opercular Muscle	774.5 ±1.07	771.4 ±1.55	770.1 ±1.35	44.8 ±1.16	53.4 ±1.68	48.5 ±1.87	729.8 ±1.94	718.1 ±2.40	721.8 ±2.08
	--NS -	- NS -		-p<.01-	- NS -		-p<.01-	- NS -	
	----- p<.05 ----			------ NS -----			------ p<.05 ----		
Mid-Dorsal Muscle	771.9 ±1.14	768.8 ±1.27	766.6 ±1.74	38.1 ±0.96	44.3 ±1.54	38.5 ±2.14	733.7 ±1.47	724.8 ±2.03	727.6 ±3.29
	- NS -	- NS -		-p<.01-	-p<.05-		-p<.01-	- NS -	
	----- p<.01 ----			------ NS ------			------- NS ------		
Caudal Muscle	772.5 ±1.89	770.7 ±3.38	769.2 ±1.59	48.5 ±0.88	50.5 ±1.19	48.6 ±1.88	725.1 ±1.36	722.6 ±2.51	720.4 ±2.47
	- NS -	- NS -		- NS -	- NS -		- NS -	- NS -	
	----- p<.05 ----			------- NS ------			------ NS -------		
Cardiac Muscle	808.8 ±1.58	807.1 ±2.68	806.2 ±2.57	215.5 ±3.43	222.0 ±4.15	240.6 ±3.58	588.4 ±3.62	589.7 ±4.63	565.6 ±5.19
	- NS -	- NS -		- NS -	-p<.01-		- NS -	-p<.01-	
	------ NS ------			----- p<.01 ----			----- p<.01 ----		
Liver	742.5 ±1.71	739.8 ±3.41	747.1 ±2.65	213.5 ±4.43	244.4 ±5.97	247.3 ±6.57	527.9 ±4.55	495.0 ±4.78	500.2 ±6.83
	- NS -	- NS -		-p<.01-	- NS -		-p<.01-	- NS -	
	------ NS ------			------ p<.01 ----			------ p<.01 ----		
Spleen	732.0 ±2.92	752.7 ±2.90	764.8 ±4.14	142.2 ±4.38	139.4 ±4.91	161.4 ±6.20	591.5 ±5.25	613.1 ±5.07	603.4 ±8.14
	-p<.01-	-p<.05-		- NS -	-p<.05-		-p<.01-	- NS -	
	------ p<.01 ----			----- p<.05 ----			------- NS ------		
Gut	771.4 ±2.92	767.7 ±4.35	763.0 ±4.70	372.8 ±8.05	413.9 ±13.72	404.5 ±13.31	398.5 ±7.78	348.5 ±14.59	358.6 ±14.33
	- NS -	- NS -		-p<.05-	- NS -		-p<.05-	- NS -	
	------ NS ------			------- NS ------			------ p<.05 -----		
Brain	807.8 ±1.72	814.4 ±2.51	809.7 ±2.09	291.2 ±7.98	343.2 ±8.49	328.4 ±9.07	515.9 ±7.51	472.5 ±7.76	482.3 ±9.96
	-p<.05-	- NS -		-p<.01-	- NS -		-p<.01-	- NS -	
	------- NS ------			------ p<.05 ----			------ p<.05 ----		

(A) Plasma

Figure 6 indicates plasma sodium, potassium and chloride concentrations and water content for thermally-acclimated rainbow trout.

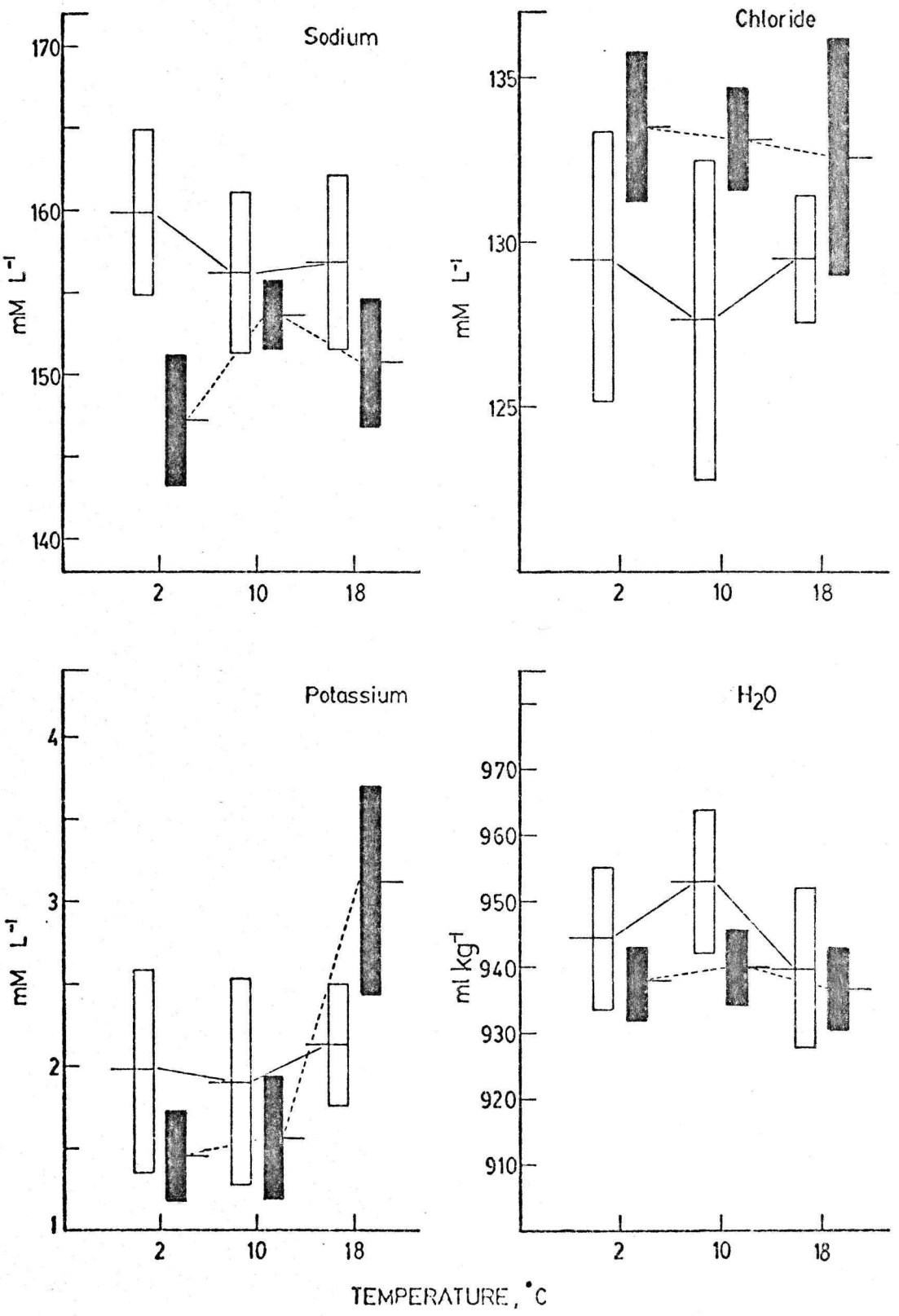
'Summer' fish displayed no significant variations in electrolyte levels or water content over the range of temperatures considered. Sodium concentrations were held between 156.2 ± 2.27 and 159.9 ± 2.31 mM l^{-1}, chloride between 127.6 ± 2.29 and 129.5 ± 0.91 mM l^{-1}, potassium between 1.90 ± 0.29 and 2.13 ± 0.17 mM l^{-1}, and water from 939.9 ± 5.73 to 953.1 ± 4.98 ml kg^{-1}.

'Fall-winter' series of fish were characterized by a somewhat different pattern of plasma composition, and were also distinguishable in terms of acclimatory responses. For example, only chloride concentration and water content were held reasonably stable over the 2° to 18°C temperature range. Plasma sodium concentrations increased significantly ($P < 0.01$) between 2° (147.2 ± 1.94 mM l^{-1}) and 10° (153.6 ± 1.01 mM l^{-1}). No significant differences were apparent between 2° and 18°C (150.7 ± 1.85 mM l^{-1}) and 10° and 18°C. Plasma potassium concentrations increased significantly ($P < 0.01$) at 18°C, rising to 3.12 ± 0.32 mM l^{-1} from the levels characterizing 2°C (1.45 ± 0.13 mM l^{-1}) and 10°C animals (1.56 ± 0.18 mM l^{-1}).

In general, these observations compare favorably with values previously reported for rainbow trout (Hickman et al., 1964; Houston et al., 1968; Murphy and Houston, 1977). Hickman et al. (1964) and Murphy and Houston (1977) reported similar values for plasma sodium, although in both studies there was some decrease in concentration with increased temperature. Increased plasma potassium concentration in 'fall-winter' trout was also reported by Houston et al. (1968) and Murphy and Houston (1977). Plasma water content was found to be relatively stable by Hickman et al. (1964).

Figure 6 Plasma electrolyte (mM l^{-1}) and water content (ml kg^{-1}) of rainbow trout acclimated to 2°, 10° and 18°C. Horizontal line represents the mean and the vertical bar is the 95% confidence interval of the mean. The white bar represents 'summer' series fish, while the dark bar represents 'fall-winter' animals.

PLASMA


Sodium
mM L⁻¹
170
160
150
140
2
10
18
Chloride
mM L⁻¹
135
130
125
2
10
18
Potassium
mM L⁻¹
4
3
2
1
2
10
18
H2O
ml kg⁻¹
970
960
950
940
930
920
910
2
10
18
TEMPERATURE, °C

The occurrence of seasonal differences in iono-regulatory status will be apparent. In 'fall-winter' fish, chloride levels were significantly higher ($P < 0.05$) than those seen in 'summer' animals, except at 18°C. In 'summer' trout, sodium concentrations were greater ($P < 0.05$) than in the 'fall' fish, except at 10°C. Plasma potassium increased sharply between 10° and 18°C in 'fall' fish; an observation confirming the results of Gordon (1959), Houston et al. (1968) and Murphy and Houston (1977). This increase in plasma potassium under conditions of elevated temperature in 'fall-winter' populations and those under 'winter' photoperiod conditions seems to be unique to salmonids. No similar changes in plasma potassium levels with temperature have been reported for fall-sampled carp (Houston and Madden, 1968; Houston et al., 1970) or for brown bullhead (Grigg, 1969), although Heinicke and Houston (1965b) reported significant elevations in plasma potassium of goldfish with warm acclimation.

Seasonal variations, notwithstanding, it will be apparent that plasma electrolyte levels are, with one exception, highly regulated despite major variations in temperature and imposition of the types of stresses previously identified. The principal exception to this generalization is, of course, the increase in plasma potassium of 'fall-winter' fish which has been consistently observed in trout, as noted earlier. However, neither the mechanism underlying this increase, nor the physiological significance, if any, of this departure from the regulated state is known.

Quite clearly, the rainbow trout must possess effective means of compensating for temperature-induced increases in water efflux, and branchial and urinary electrolyte losses. Presumably this involves either

(i) compensatory decrease in water and ion permeability, or

(ii) compensatory increases in electrolyte absorption or reabsorption.

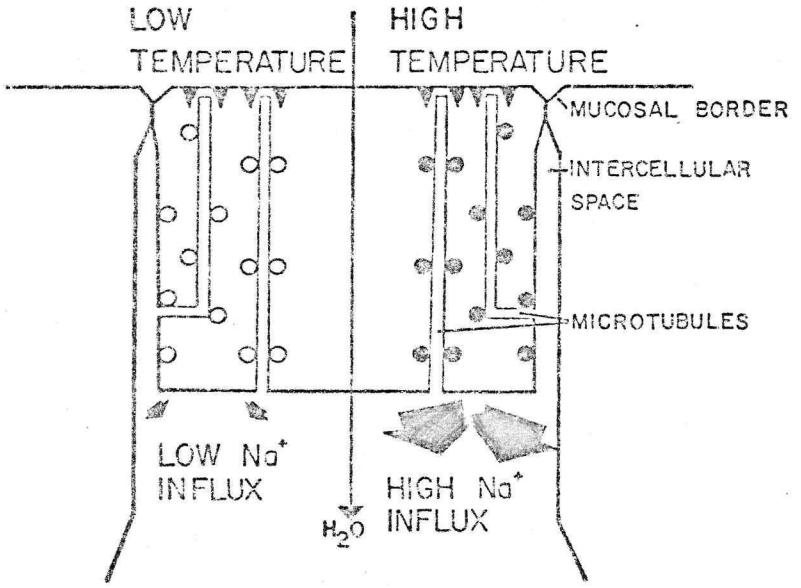
However, the fact that temperature effects on rates of oxygen consumption, ventilatory flow and diffusional water influx are comparable (Evans, 1969; Isaia, 1972; Motais and Isaia, 1972) suggests that reductions in lamellar water permeability do not occur or do not play a major role in the compensatory process. There is some evidence that ionic permeabilities may be reduced at higher temperatures, since variations in ion influx were below the corresponding changes in the rate of endosmosis (Maetz, 1972; Cameron, 1976). In any case, there must be some sort of regulatory system at work. For example, Evans (1969) has shown that water influx in some freshwater fishes increases twofold with a 10° increase in temperature (i.e., $Q_{10} \simeq 2.0$). If this is also the case for trout, then the homeostatic system must be very effective since plasma water content varies by less than 1% in the trout analyzed. Recent studies indicate that branchial absorption processes are likely of particular importance under these circumstances.

The 'chloride' cell model of branchial ion uptake and exchange (Maetz, 1971) illustrates the importance of the role of the branchial epithelium in these processes. Sodium and chloride transport are considered to be independent, but at the same time there is an inter-relationship of ionic exchange. A slightly modified model of Maetz's 'chloride' cell (as redrawn from McCarty, 1977) is shown in Figure 7, and takes into account the effect of temperature on the various exchange and transport components of the system. The independent Na^+/NH_4^+ or H^+ and Cl^-/HCO_3^- exchanges are located on the mucosal border, on the water-cell boundary. (Na^+-K^+)-ATPase also plays a large role in Na^+ uptake, although as indicated in Figure 7, this is of more importance at higher temperatures than at low temperatures. In addition to the Cl^-/HCO_3^- exchange the existence of a chloride pump, which might be

Figure 7a A model of chloride cell function at high (lower illustration) and low (upper illustration) temperature. The location of the various processes is indicated on the figure. Substantial involvement is indicated by solid lines and circles, whereas limited involvement is indicated by broken lines and open circles.

7b A model illustrating how chloride cell structures, (Na^+-K^+)-ATPase distribution, and changes in the activity of this enzyme could be used to explain the known changes in sodium uptake (indicated by arrows) at low (left) and high (right) temperatures.

A PROPOSED MODEL RELATING CHLORIDE CELL STRUCTURE AND ENZYME ACTIVITIES



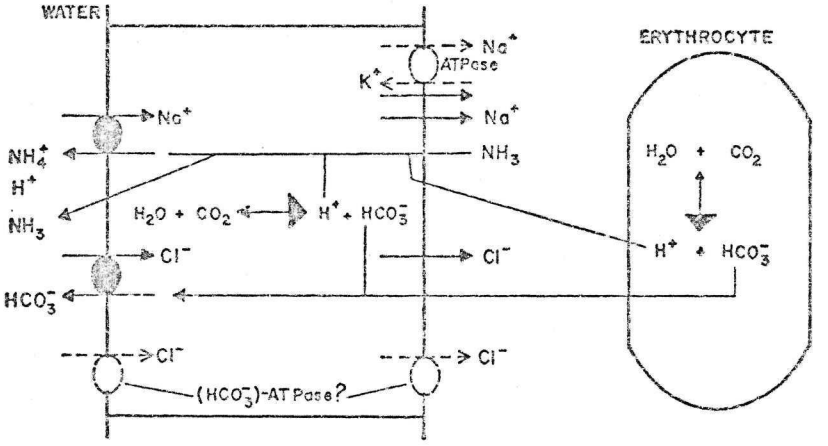
▼ Na^+/NH_4^+-H^+ EXCHANGE, FUELED BY CARBONIC ANHYDRASE

○ INACTIVE (Na^+-K^+)-ATPase

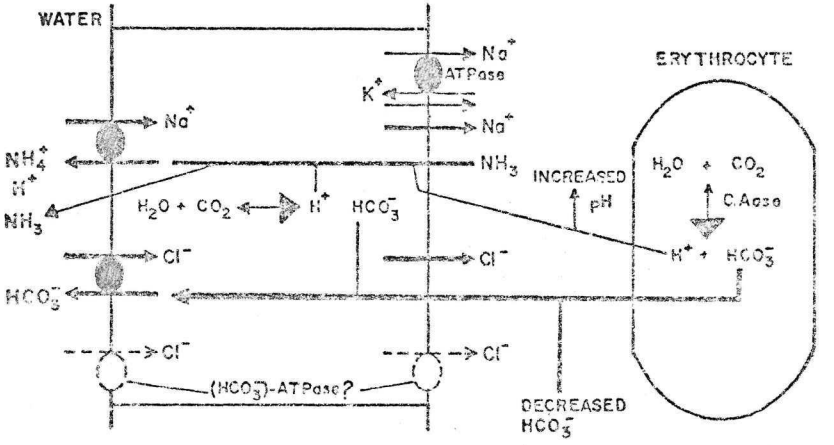
◉ ACTIVE (Na^+-K^+)-ATPase

A REVISED MODEL OF CHLORIDE CELL FUNCTION

LOW TEMPERATURES



HIGH TEMPERATURES



(HCO_3^-)-ATPase (known to exist in trout gills (Watson, 1977)), is suggested. Branchial and blood carbonic anhydrase, which by catalyzing the ultimate formation of HCO_3^- and H^+ from CO_2 and H_2O provide counter-exchange ions for both exchange processes, seem to be of greater importance at low temperatures. Both gill (Na^+-K^+)-ATPase and erythrocytic carbonic anhydrase activity have been shown to increase with temperature (McCarty and Houston, 1977; Smeda and Houston, 1978), and therefore provide for the increased ion uptake necessary at elevated temperatures in freshwater fish.

(B) Epaxial Muscle

1. Water Content and Distribution

Water content and distribution data are summarized in Figures 8, 9 and 10 a to c, and Table 8 ('summer') and Table 10 ('fall').

'Summer' Series

Tissue water content (g kg^{-1}) was unchanged over the temperature range employed, except in the case of caudal samples (Figures 8, 9, 10a). Here water content increased ($P < 0.05$) between 2° and 10°.

For reasons discussed in the previous section, extracellular phase volume was estimated in terms of the Cl/K space. This volume increased with temperature (Table 8, Figs. 8, 9, 10c), with maximum values occurring at 10°C. Although the changes encountered were not significant, there was also a clear trend towards larger extracellular volume in caudal muscle.

By reference to concommitant changes in cellular phase volume (Figs. 8, 9 and 10b), it would appear that water moved from the cellular phase into the extracellular phase, since overall tissue water content was not significantly altered. Such a shift appeared to be significant in the case of post-opercular muscle (Table 8) but not with respect to the mid-dorsal and

Figure 8 Water and electrolyte parameters for post-opercular muscle of rainbow trout acclimated to 2°, 10° and 18°C are displayed

8a Water content

8b Cellular phase volume

8c Extracellular phase volume (Cl/K space)

8d Tissue sodium concentrations

8e Tissue chloride concentrations

8f Tissue potassium concentration

8g Cell sodium concentrations

8h Cell chloride concentrations

8i Cell potassium concentrations

8j Sodium equilibrium potential (E_{Na})

8k Chloride equilibrium potential (E_{Cl})

8l Potassium equilibrium potential (E_{K})

Horizontal line in all figures represents the mean, while the vertical bar is the 95% confidence interval of the mean.

Light bar--'summer' series

Dark bar--'fall-winter' series

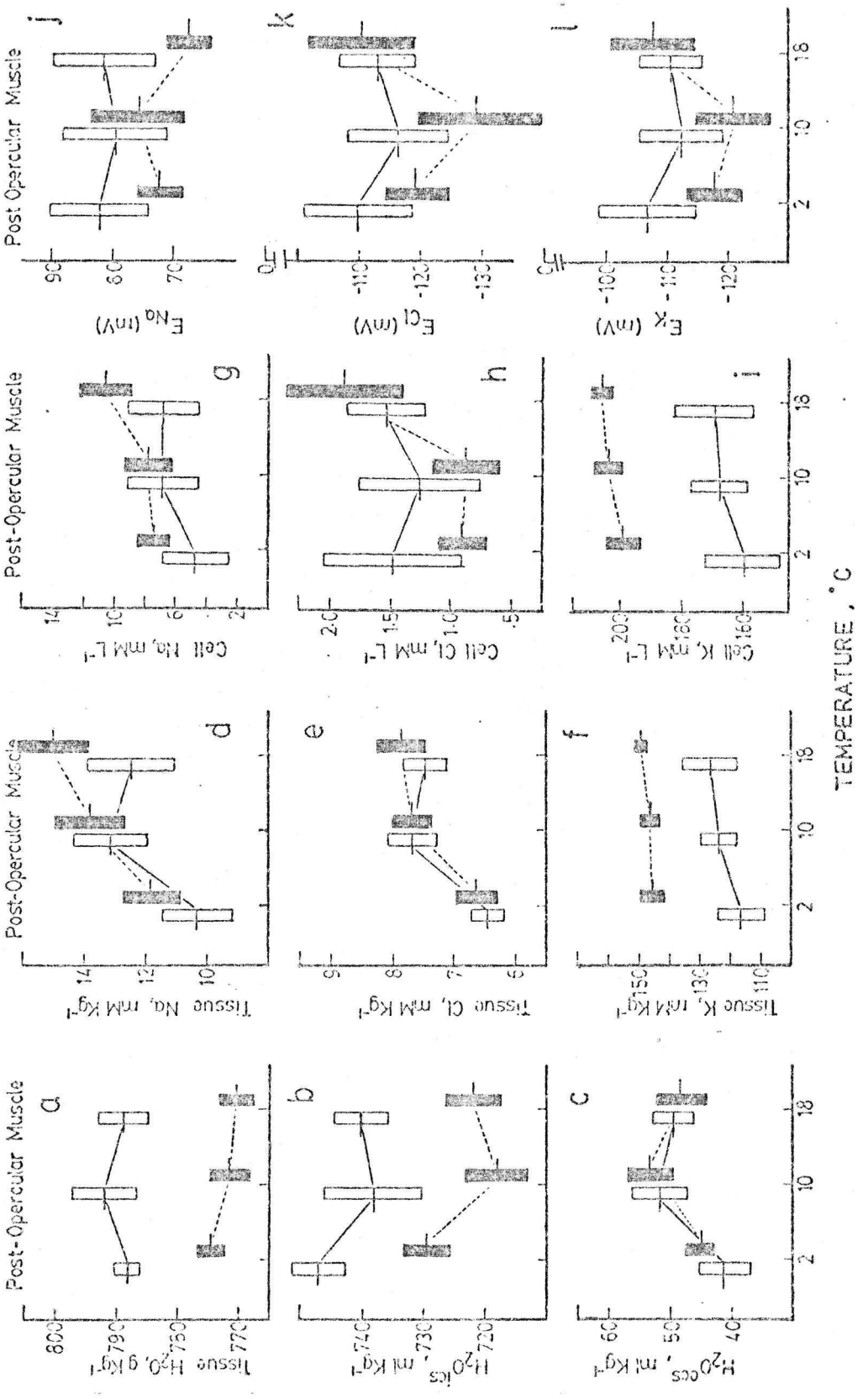

Post-Opercular Muscle
a
b
c
d
e
f
g
h
i
j
k
l
Tissue H2O, g kg-1
H2Oics, ml kg-1
H2Occs, ml kg-1
Tissue Na, mM kg-1
Tissue Cl, mM kg-1
Tissue K, mM kg-1
cell Na, mM L-1
cell Cl, mM L-1
cell K, mM L-1
ENa (mV)
ECl (mV)
EK (mV)
TEMPERATURE, °C

Fig. 8

Figure 9 Water and electrolyte parameters for mid-dorsal muscle of rainbow trout acclimated to 2°, 10° and 18°C.

9a Water content

9b Cellular phase volume

9c Extracellular phase volume (Cl/K space)

9d Tissue sodium concentrations

9e Tissue chloride concentrations

9f Tissue potassium concentrations

9g Cell sodium concentrations

9h Cell chloride concentrations

9i Cell potassium concentrations

9j Sodium equilibrium potential (E_{Na})

9k Chloride equilibrium potential (E_{Cl})

9l Potassium equilibrium potential (E_{K})

Horizontal line represents the mean; the vertical bar is the 95% confidence interval of the mean. Light bar--'summer' series trout; dark bar--'fall-winter' series fish.

Fig. 9

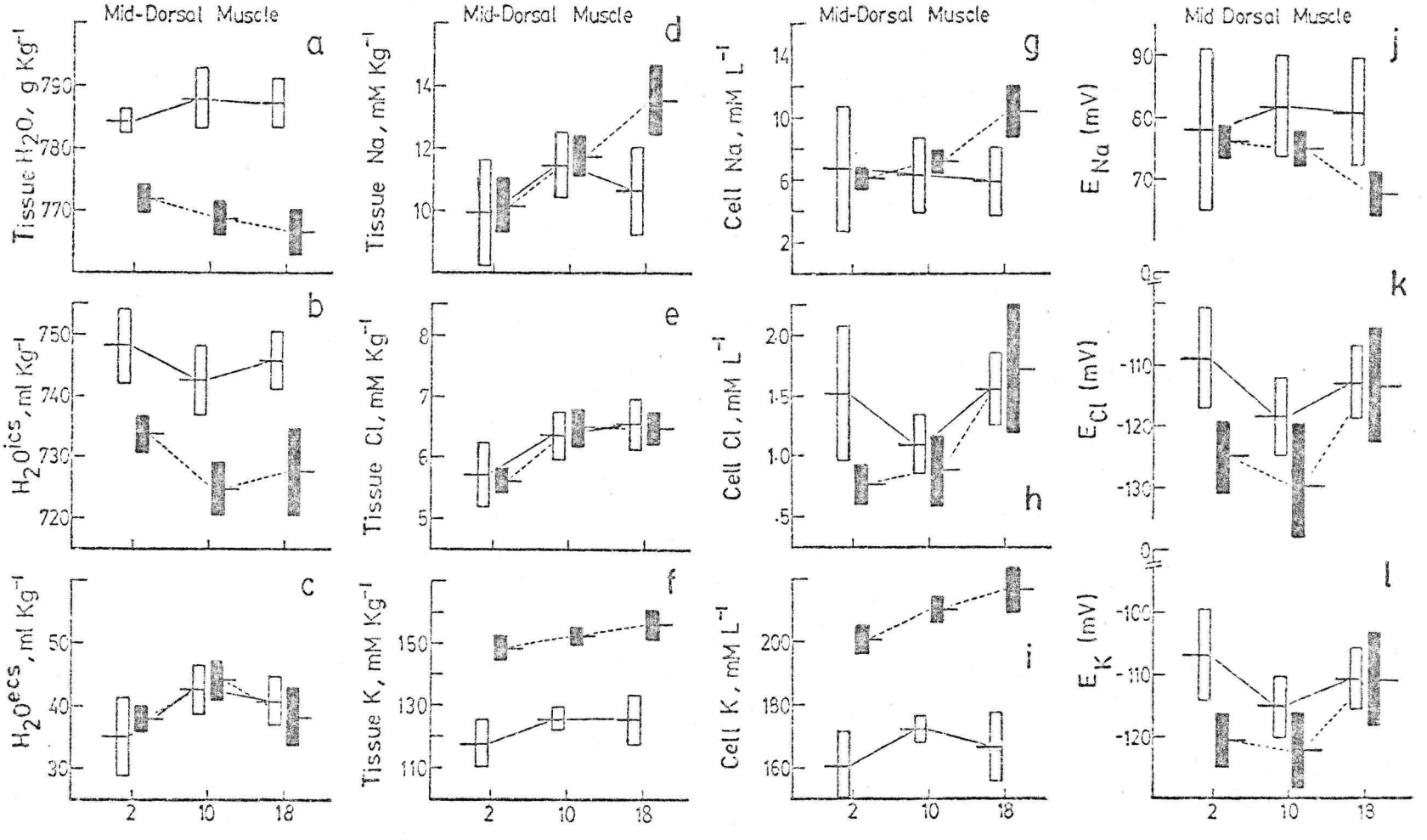

Mid-Dorsal Muscle
a
Tissue H_2O, g Kg^{-1}
790
780
770
b
H_2O^{ics}, ml Kg^{-1}
750
740
730
720
c
H_2O^{ecs}, ml Kg^{-1}
50
40
30
2
10
18
Mid-Dorsal Muscle
d
Tissue Na, mM Kg^{-1}
14
12
10
e
Tissue Cl, mM Kg^{-1}
8
7
6
5
f
Tissue K, mM Kg^{-1}
150
130
110
2
10
18
Mid-Dorsal Muscle
g
Cell Na, mM L^{-1}
14
10
6
2
h
Cell Cl, mM L^{-1}
2.0
1.5
1.0
.5
i
Cell K, mM L^{-1}
200
180
160
2
10
18
Mid Dorsal Muscle
j
E_{Na} (mV)
90
80
70
k
E_{Cl} (mV)
0
-110
-120
-130
l
E_K (mV)
0
-100
-110
-120
2
10
18
TEMPERATURE , °C

Figure 10 Water and electrolyte parameters for caudal muscle of rainbow trout acclimated to 2°, 10° and 18°C.

10a Water content

10b Cellular phase volume

10c Extracellular phase volume

10d Tissue sodium concentrations

10e Tissue chloride concentrations

10f Tissue potassium concentrations

10g Cell sodium concentrations

10h Cell chloride concentrations

10i Cell potassium concentrations

10j Sodium equilibrium potentials (E_{Na})

10k Chloride equilibrium potentials (E_{Cl})

10l Potassium equilibrium potential (E_K)

Horizontal bar represents the mean, while the vertical bar is the 95% confidence interval of the mean. Light bar--'summer' series fish; dark bar--'fall-winter' fish.

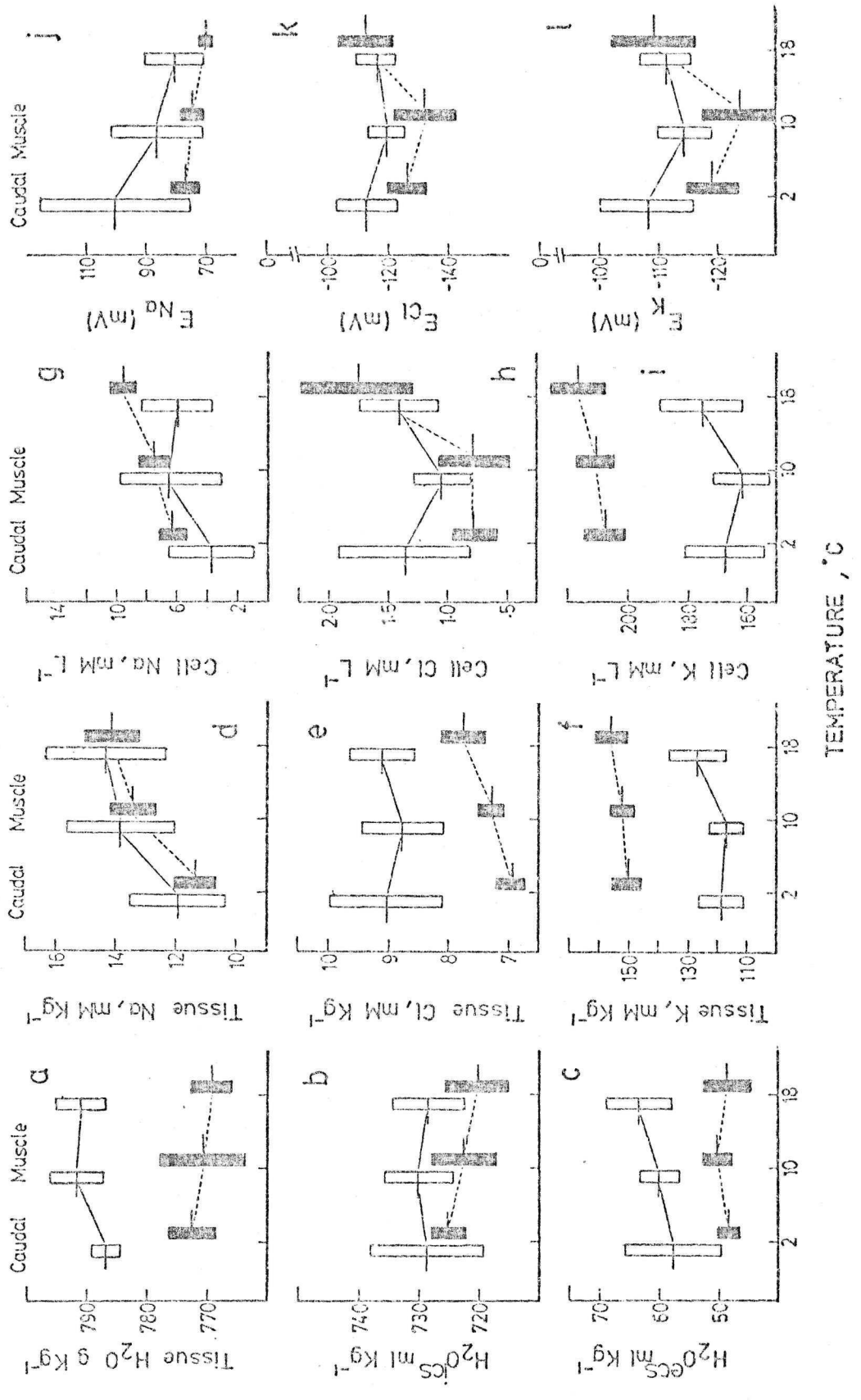

Caudal Muscle
a
b
c
d
e
f
g
h
i
j
k
l
Tissue H2O g kg-1
H2O ECS ml kg-1
H2O ICS ml kg-1
Tissue Na, mM kg-1
Tissue Cl, mM kg-1
Tissue K, mM kg-1
Cell Na, mM L-1
Cell Cl, mM L-1
Cell K, mM L-1
ENa (mV)
ECl (mV)
EK (mV)
TEMPERATURE, °C

Fig. 10

caudal muscle sites.

'Fall-Winter'Series

Cold acclimation (2°C) was associated with an increase in water content (Figs. 8, 9 and 10a, Table 9), a situation which contrasts sharply with that encountered in 'summer' trout. Tissue hydration at lower temperatures is, however, one of the more consistent features of acclimatory response, and has been encountered in a number of species (Gordon, 1959; Houston et al., 1968, 1970; see also Houston, 1973, for review).

Extracellular phase volume tended to be higher at 10°C than at either 2° or 18°C, although these variations were not always significant, and were less apparent in caudal muscle than in post-opercular or mid-dorsal samples (Figs. 8, 9 and 10c; Table 9).

Cellular phase volume tended to decrease between 2° and 10°C, and in general variations in the volume of this phase were a mirror image of those in ECPV (Figs. 8, 9 and 10b). It appears that water moved out of the cellular phase with increasing temperature since both tissue water and ICPV decreased under these conditions, while ECPV tended to increase. Again, this represents one of the more common features of thermoacclimatory response (Houston, 1973).

Also of interest, in terms of water distribution, were the differences among muscle sites. Overall tissue water levels were generally similar at all three sites; ECPV, on the other hand, differed. Thus, mid-dorsal muscle was characterized by a lower ECPV than either post-opercular or caudal muscle at any given acclimation temperature. This was also encountered in 'summer' fish. In that case, however, caudal muscle rather than mid-dorsal displayed the smallest ECPV.

Seasonal Considerations

Seasonal differences in water content and distribution were apparent. Water content was significantly higher ($P < 0.01$) in 'summer' than in 'fall' fishes at all muscle sites. This was also true of the ICPV of 'summer' post-opercular and mid-dorsal muscle samples. Such differences amounted to only about 2%. However, the ECPV of both groups were very similar, and showed corresponding changes with temperature, the only exception being caudal muscle.

2. Tissue Electrolyte Levels

Data for overall tissue electrolyte levels are summarized in Table 7 ('summer') and Table 9 ('fall'), and graphically presented in Figures 8, 9, and 10 d to f.

'Summer' Series

Sodium: Post-opercular muscle sodium concentration (mM kg^{-1} wet wt.) increased between 2° and 10° ($P < 0.01$) and 2° and 18°C ($P < 0.05$) (Fig. 8d, open bars). There was, however, no significant variation in the sodium content of either mid-dorsal (Fig. 9d, open bars) or caudal muscle samples (Fig. 10d, open bars). In addition, the sodium concentrations at the mid-dorsal muscle site were lower than those of post-opercular and caudal muscle (Table 7).

Chloride: Both post-opercular and mid-dorsal muscle chloride concentrations (mM kg^{-1} wet wt) increased ($P < 0.01$) with temperature, the largest increment being seen between 2° and 10°. No significant differences were apparent in 10° and 18°C animals (Figs 8e and 9e). Caudal muscle chloride content did not vary significantly with temperature, although it was higher than that in either of the other two samples (Fig. 10e, Table 7).

Potassium: In general, muscle potassium concentration appeared to be stable with acclimation temperature (Figs. 8, 9 and 10f). However, there was a significant increase in concentration ($P < 0.05$) between 2° and 10°C at the mid-dorsal muscle site.

'Fall-Winter' Series

Sodium: A somewhat different pattern of change with acclimation was encountered in the 'fall-winter' series of fish (Table 9). Tissue sodium concentration increased with temperature at each of the three muscle sites sampled (Figs. 8, 9 and 10d). For example, the sodium content of each increased significantly ($P < 0.01$) between 2° and 10°, and 2° and 18°C. However, only mid-dorsal muscle showed a significant increase ($P < 0.01$) between 10° and 18°C.

Chloride: In the case of chloride, the changes in post-opercular and mid-dorsal were essentially similar to those of 'summer' fish (Figs. 8 and 9c). Significant increases ($P < 0.01$) took place between 2° and 10°C, but no further increases were seen between 10° and 18°C. In caudal muscle only, a significant increase in chloride ($P < 0.05$) also took place between 10° and 18°C (Fig. 10e, Table 9).

Potassium: In general, the potassium content of muscle remained relatively stable (Figs. 8 and 10f). However, potassium concentration of mid-dorsal muscle increased significantly ($P < 0.05$) between 2 and 18°C (Fig. 9f).

Seasonal Considerations

As was true of water content, tissue electrolyte levels exhibited several differences which appeared to be attributable to season. For example, tissue sodium concentrations of 'fall-winter' specimens were significantly higher ($P < 0.01$) than those of 'summer' animals at 18°C. Potassium concentrations of 'fall' muscle were some 10 to 30 mM kg^{-1} in excess of those of the 'summer' series trout at any given acclimation

temperature. By contrast, chloride levels were similar in both concentration and thermoacclimatory response in both seasonal groups. Caudal muscle, however, was an exception to this; chloride content in the 'summer' series of fish were higher than those of the 'fall' animals.

3. Cellular Electrolyte Levels.

Cellular electrolyte concentrations in mM l^{-1}, cell water, as calculated using the Cl/K space estimate of ECPV, are summarized in Tables 11 (summer) and 12 (fall), and Figures 8, 9 and 10 g to i, for each of the three muscle sites.

'Summer' Series

As expected, potassium was the predominant electrolyte of the cellular compartment. Sodium and chloride, which are predominantly extracellular ions, were also present in significant amounts. In general, sodium concentrations exceeded those of chloride (Table 11).

In general, no significant variation in cellular electrolyte levels attributable to acclimation were encountered in the summer fish. There were only two exceptions to this generalization. Cellular chloride concentration of mid-dorsal muscle increased significantly ($P < 0.05$) between 10° and 18°C, while cellular potassium levels at this muscle site increased ($P < 0.05$) between 2° and 10°C. The variations observed, although non-significant, in cellular electrolyte levels with temperature in general corresponded with those observed for overall tissue electrolyte levels.

'Fall-Winter' Series

A quite different situation was seen in the 'fall-winter' series of fish. Cellular potassium concentrations were maintained at reasonably constant levels, except at the mid-dorsal muscle site where potassium increased significantly ($P < 0.01$) with temperature (Table 12, Figs. 8, 9 and 10i). Both sodium and chloride, on the other hand, increased signifi-

TABLE 11. Cellular electrolyte levels (mM/litre cellwater) in tissues of rainbow trout (summer series) acclimated to 2, 10 and 18 C. Reported as mean ±1 standard error of the mean. Sample sizes are as reported in Table 7.

	SODIUM			CHLORIDE			POTASSIUM		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Postopercular Muscle	4.68 ±0.93	6.85 ±1.06	6.82 ±1.07	1.48 ±0.26	1.26 ±0.23	1.54 ±0.15	159.97 ±5.81	167.99 ±4.18	169.72 ±5.97
	-- NS --	-- NS --		-- NS --	-- NS --		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Middorsal Muscle	6.75 ±1.79	6.27 ±1.09	5.95 ±1.01	1.52 ±0.25	1.09 ±0.11	1.56 ±0.14	160.09 ±5.74	172.13 ±1.98	166.66 ±5.04
	-- NS --	-- NS --		-- NS --	--p<.05--		--p<.05--	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Caudal Muscle	3.72 ±1.19	6.43 ±1.49	5.99 ±1.08	1.36 ±0.25	1.05 ±0.10	1.41 ±0.15	167.61 ±6.11	161.97 ±4.43	175.39 ±6.55
	-- NS --	-- NS --		-- NS --	-- NS --		-- NS --	-- NS --	
	------- NS -----			-------- NS --------			-------- NS --------		
Cardiac Muscle	30.29 ±6.18	27.47 ±4.22	19.69 ±5.16	8.53 ±0.39	8.12 ±0.19	9.48 ±0.46	120.44 ±8.29	121.57 ±9.24	129.37 ±11.31
	-- NS --	-- NS --		-- NS --	--p<.01--		-- NS --	-- NS --	
	-------- NS --------			-------- NS --------			-------- NS --------		
Liver	----	----	----	31.01 ±2.69	40.76 ±1.76	41.59 ±2.37	129.54 ±13.18	187.07 ±8.27	159.09 ±5.56
				--p<.05--	-- NS --		--p<.01--	--p<.01--	
				------- p<.05 ------			------- p<.05 ------		
Gut	----	----	----	6.52 ±0.43	8.18 ±0.58	7.78 ±0.67	109.60 ±11.25	126.76 ±11.53	92.02 ±9.82
				-- NS --	-- NS --		-- NS --	-- NS --	
				-------- NS --------			-------- NS --------		
Brain	49.75 ±10.93	58.29 ±4.94	51.36 ±3.70	8.73 ±0.49	9.19 ±0.38	10.27 ±0.38	147.41 ±11.37	134.57 ±4.74	177.49 ±10.48
	-- NS --	-- NS --		-- NS --	--p<.05--		-- NS --	--p<.01--	
	-------- NS --------			------- p<.05 -------			-------- NS --------		

TABLE 12. Cellular electrolyte levels (mM/litre cell water) in tissues of rainbow trout (fall series) acclimated to 2, 10 and 18 C. Reported as mean ± 1 standard error of the mean. Sample sizes are as reported in Table 8.

	SODIUM			CHLORIDE			POTASSIUM		
	2 C	10 C	18 C	2 C	10 C	18 C	2 C	10 C	18 C
Postopercular Muscle	7.41 ±0.52	7.82 ±0.75	10.65 ±0.81	0.97 ±0.09	0.87 ±0.13	1.88 ±0.23	199.27 ±2.73	203.33 ±2.26	206.86 ±1.53
	-- NS --	--p<.05-		-- NS --	--p<.01-=		-- NS --	-- NS --	
	------- p<.01 ------			------- p<.01 -------			-------- NS --------		
Middorsal Muscle	6.06 ±0.32	7.21 ±0.36	10.43 ±0.76	0.76 ±0.07	0.87 ±0.14	1.72 ±0.25	200.77 ±2.20	210.29 ±1.92	216.53 ±3.33
	--p<.05 --	--p<.01-=		-- NS ---	--p<.01-=		--p<.01-	-- NS --	
	------- p<.01 -------			------- p<.01 -------			------- p<.01 ------		
Caudal Muscle	6.27 ±0.44	7.49 ±0.49	9.56 ±0.41	0.77 ±0.09	0.78 ±0.14	1.76 ±0.22	207.88 ±3.23	210.79 ±2.98	216.62 ±4.19
	-- NS --	--p<.01-=		-- NS ---	--p<.01-=		-- NS --	-- NS --	
	------- p<.01 -------			------- p<.01 -------			-------- NS --------		
Cardiac Muscle	20.04 ±2.02	20.12 ±2.26	20.94 ±2.09	5.44 ±0.30	5.55 ±0.14	6.34 ±0.16	65.42 ±3.49	79.28 ±4.79	65.92 ±3.74
	-- NS --	-- NS --		-- NS --	--p<.01-=		--p<.05-	--p<.05-	
	-------- NS --------			------- p<.01 -------			-------- NS --------		
Liver	----	----	-----	17.64 ±1.26	24.29 ±1.34	32.52 ±2.96	218.26 ±5.20	247.21 ±4.72	231.76 ±5.70
				--p<.01-	-- NS --		--p<.01-	-- NS --	
				------- p<.01 ------			-------- NS --------		
Spleen	----	----	-----	27.02 ±1.09	37.72 ±0.98	43.53 ±1.56	165.59 ±6.59	163.90 ±6.28	156.09 ±6.59
				--p<.01-=	--p<.01-=		-- NS --	-- NS --	
				-------p<.01 -------			-------- NS --------		
Gut	----	----	-----	14.14 ±0.55	18.94 ±1.50	17.34 ±1.16	113.09 ±6.04	149.54 ±9.79	113.97 ±6.64
				--p<.05-	-- NS --		--p<.01-	--p<.01-	
				------ p<.01 ------			-------- NS --------		
Brain	41.57 ±3.42	45.87 ±3.93	50.88 ±2.19	8.48 ±0.35	10.79 ±0.49	10.19 ±0.44	62.02 ±2.99	78.15 ±3.52	69.71 ±5.20
	-- NS --	-- NS --		--p<.01-=	-- NS --		--p<.01-	-- NS --	
	------- p<.05 ------			------- p<.01 ------			-------- NS --------		

cantly with temperature (Figs. 8, 9 and 10 g and h). The largest increment in each case was between 10° and 18°, and 2° and 18°C.

Cell Na^+ concentrations were comparable to those reported for rainbow trout by Hickman *et al.* (1964) and Murphy and Houston (1977). Cell Cl^- was lower, and cell K^+ higher than values reported by the above authors.

Seasonal Considerations

As was true of tissue electrolyte levels, cellular electrolyte concentrations also exhibited a number of seasonal variations. Cell cation levels tended to be higher in 'fall' than in 'summer' fish. For example, potassium concentrations were some 40 mM l^{-1} in excess of the animals of the 'summer' series at any given acclimation temperature (Figs. 8, 9 and 10i). 'Fall' sodium concentrations were higher than 'summer' levels only at 18°C. Chloride concentrations, by contrast, tended to be lower in 'fall' than in 'summer' fish particularly at the lower acclimation temperatures (Figs. 8, 9 and 10h).

The pattern of response was also different, with 'fall' sodium and chloride concentrations increasing with temperature and 'summer' concentrations remaining relatively stable.

4. Effect of Temperature on Electrolyte Distribution

An attempt was made to clarify the relationship between cellular electrolyte concentration and overall tissue electrolyte concentrations. By rearranging the formula used to calculate intracellular ion concentrations (Chan and Wong, 1977), the following equation was obtained:

$$Cm = (Cec \cdot ECPV) + (Cic \cdot ICPV)$$

where: Cm = concentration of electrolyte per kg wet weight

Cec = concentration of electrolyte in extracellular compartment;

essentially the plasma concentration (mM l^{-1})

Cic = concentration of electrolyte in intracellular compartment; (mM l^{-1})

ECPV = extracellular phase volume, l kg^{-1}

ICPV = intracellular phase volume, l kg^{-1}

Using this formula, the amount of electrolyte in the extracellular fluid volume of the muscle (Cec•ECPV) and in the intracellular fluid (Cic•ICPV) can be determined. The results of this calculation, carried out using mean values only, are summarized in Table 13 ('summer') and Table 14 ('fall').

As can be seen from these tables, potassium was indeed the major cellular electrolyte with 99.9% of the tissue potassium located in the cellular compartment, and only about 0.1% in the extracellular phase. This relationship did not change with temperature in either series of fish. Sodium and chloride, on the other hand, exhibited more variation in relation to acclimation. However, as these calculations were carried out on mean values it was not possible to ascertain the significance of the differences observed.

Cellular sodium in 'fall-winter' fish constituted 40 to 50% of the total tissue sodium; sodium in the extracellular phase was about 50 to 60% of the total. In 'summer' fish, however, extracellular sodium made up 60 to 70% of the total. This can be correlated with the greater plasma concentration of sodium in summer fish, since the ECPV of muscle in both series of fish was comparable.

The distribution of chloride between the intracellular and extracellular compartments in both 'summer' and 'fall' muscle was similar, with 80 to 90% of total muscle chloride being in the extracellular compartment.

TABLE 13. Distribution of electrolytes between extracellular fluid volume (ECFV) and intracellular fluid volume (ICFV). Na_e^+, Cl_e^- and K_e^+ are concentrations (mM/kg) in ECFV. Na_i^+, Cl_i^- and K_i^+ are concentrations (mM/kg) in ICFV. % Total is the percentage of the total tissue concentration attributable to either the ECFV or ICFV concentrations of each electrolyte. The reported values have been calculated from mean values from summer series fish.

		Na_e^+	% T	Na_i^+	% T	Cl_e^-	% T	Cl_i^-	% T	K_e^+	% T	K_i^+	% T
Post-	2°	6.6	65.0	3.5	35.0	5.3	83.0	1.10	17.0	0.08	0.1	119.5	99.9
Opercular	10°	8.1	61.0	5.1	39.0	6.6	88.0	0.93	12.0	0.10	0.1	124.0	99.9
Muscle	18°	7.8	60.0	5.1	40.0	6.4	85.0	1.10	15.0	0.11	0.1	125.6	99.9
Mid-	2°	5.6	52.0	5.1	48.0	4.5	80.0	1.10	20.0	0.07	0.1	119.8	99.9
Dorsal	10°	6.7	59.0	4.7	41.0	5.5	87.0	0.81	13.0	0.08	0.1	127.8	99.9
Muscle	18°	6.4	59.0	4.4	41.0	5.3	82.0	1.16	18.0	0.09	0.1	124.3	99.9
Caudal	2°	9.3	77.0	2.7	23.0	7.5	88.0	0.99	12.0	0.11	0.1	122.1	99.9
Muscle	10°	9.4	67.0	4.7	33.0	7.7	91.0	0.77	9.0	0.11	0.1	118.2	99.9
	18°	10.0	69.0	4.4	31.0	8.2	89.0	1.00	11.0	0.13	0.1	127.8	99.9
Cardiac	2°	48.1	76.0	15.6	24.0	38.9	90.0	4.4	10.0	0.59	0.9	62.1	99.1
Muscle	10°	46.0	76.0	14.2	24.0	37.6	90.0	4.2	10.0	0.56	0.9	62.8	99.1
	18°	50.9	84.0	9.8	16.0	42.1	90.0	4.7	10.0	0.69	1.0	64.1	99.0
Liver	2°	--	--	--	--	25.6	60.0	17.2	40.0	0.39	0.5	72.0	99.5
	10°	--	--	--	--	31.7	60.0	20.9	40.0	0.47	0.5	95.9	99.5
	18°	--	--	--	--	27.0	54.0	22.7	46.0	0.44	0.5	86.9	99.5
Gut	2°	--	--	--	--	31.4	90.0	3.5	10.0	0.59	1.0	59.6	99.0
	10°	--	--	--	--	35.7	90.0	4.1	10.0	0.53	0.8	63.6	99.2
	18°	--	--	--	--	35.0	90.0	4.1	10.0	0.58	1.2	47.9	98.8
Brain	2°	48.1	66.0	25.2	34.0	38.9	90.0	4.4	10.0	0.59	0.8	74.7	99.2
	10°	49.4	63.0	28.4	37.0	40.4	90.0	4.5	10.0	0.60	0.9	65.7	99.1
	18°	53.4	69.0	24.4	31.0	44.0	90.0	4.9	10.0	0.71	0.8	84.2	99.2

TABLE 14. Distribution of electrolytes between extracellular fluid volume (ECFV) and intracellular fluid volume (ICFV). Na_e^+, Cl_e^- and K_e^+ are concentrations (mM/kg) in ECFV. Na_i^+, Cl_i^- and K_i^+ are concentrations (mM/kg) im ICFV. % T is the percentage of the total tissue concentrations attributable to either the ECFV or ICFV concentrations of each electrolyte. The reported values have been calculated from mean values from fall series fish.

		Na_e^+	% T	Na_i^+	% T	Cl_e^-	% T	Cl_i^-	% T	K_e^+	% T	K_i^+	% T
Post-	2°	6.5	55.0	5.4	45.0	6.0	90.0	0.71	10.0	0.07	0.1	145.5	99.9
Opercular	10°	8.2	59.0	5.6	41.0	7.1	92.0	0.60	8.0	0.08	0.1	146.4	99.9
Muscle	18°	7.3	49.0	7.7	51.0	6.4	82.0	1.40	18.0	0.15	0.1	148.8	99.9
Mid-	2°	5.6	56.0	4.4	44.0	5.1	88.0	0.71	12.0	0.06	0.1	147.3	99.9
Dorsal	10°	6.8	57.0	5.2	43.0	5.9	90.0	0.63	10.0	0.07	0.1	152.4	99.9
Muscle	18°	5.8	43.0	7.6	57.0	5.1	80.0	1.30	20.0	0.12	0.1	157.5	99.9
Caudal	2°	7.1	61.0	4.6	39.0	6.5	92.0	0.55	8.0	0.07	0.1	150.4	99.9
Muscle	10°	7.8	59.0	5.4	41.0	6.7	92.0	0.56	8.0	0.08	0.1	152.3	99.9
	18°	7.3	51.0	6.9	49.0	6.5	83.0	1.30	17.0	0.15	0.1	156.0	99.9
Cardiac	2°	31.7	73.0	11.8	27.0	28.8	90.0	3.2	10.0	0.31	0.8	38.5	99.2
Muscle	10°	34.1	73.0	12.3	27.0	29.6	90.0	3.3	10.0	0.35	0.8	46.8	99.2
	18°	36.3	75.0	11.8	25.0	31.9	90.0	3.6	10.0	0.75	2.0	37.3	98.0
Liver	2°	--	--	--	--	28.5	75.0	9.3	25.0	0.31	0.3	115.2	99.7
	10°	--	--	--	--	32.5	73.0	12.0	27.0	0.38	0.3	122.4	99.7
	18°	--	--	--	--	32.8	67.0	16.3	33.0	0.77	0.7	115.9	99.3
Spleen	2°	--	--	--	--	19.0	54.0	16.0	46.0	0.21	0.2	97.9	99.8
	10°	--	--	--	--	18.6	45.0	23.1	55.0	0.22	0.2	100.5	99.8
	18°	--	--	--	--	21.4	45.0	26.3	55.0	0.50	0.5	94.2	99.5
Gut	2°	--	--	--	--	49.8	90.0	5.6	10.0	0.54	1.2	45.1	98.8
	10°	--	--	--	--	55.1	89.0	6.6	11.0	0.65	1.2	52.1	98.8
	18°	--	--	--	--	53.6	90.0	6.2	10.0	1.26	3.0	40.9	97.0
Brain	2°	42.9	67.0	21.4	33.0	38.9	90.0	4.4	10.0	0.42	1.3	32.0	98.7
	10°	52.7	71.0	21.7	29.0	45.7	90.0	5.1	10.0	0.54	1.4	36.9	98.6
	18°	49.5	67.0	24.5	33.0	43.5	94.0	3.0	6.0	1.02	3.0	33.6	97.0

5. Nernst Equilibrium Potentials

Nernst equilibrium potentials for each ion were calculated using the Nernst relationship presented in the Methods section. These (E_{Na}, E_K and E_{Cl}) are presented for each muscle site in Figures 8, 9 and 10 j to 1. The reversal of sign for E_{Na}, compared to E_K and E_{Cl} is important, as it illustrates that the interior is positive with respect to the exterior for sodium, and the reverse for chloride and potassium.

The equilibrium potential of each ion remained remarkably stable in the 'summer' series fish, despite reported variations in concentrations with temperature. The response of these potentials to acclimation in 'fall' fish was somewhat different. E_{Na} remained relatively stable in post-opercular and caudal muscle, but decreased towards zero in mid-dorsal muscle at 18°C (Figs. 8, 9 and 10j). E_K and E_{Cl} responded similarly, decreasing in negativity at 18°C (Figs. 8, 9 and 10k, 1).

6. Discussion

It will be apparent from the preceding sections that thermal acclimation is associated with a number of alterations in the water-electrolyte status of rainbow trout skeletal muscle.

Cold acclimation was accompanied by decreased tissue and cellular levels of sodium and chloride. Cellular potassium also declined under these circumstances. Moreover, these variations were most noticeable in the 'fall' series of fish. Muscle water content in 'fall' fish tended to increase with cold acclimation, while that of'summer'fish did not vary significantly. Warm acclimation (18°C) was, of course, coupled with significant increases in tissue concentrations of sodium and chloride in both seasonal groups, and a major rise in these electrolytes in the cellular phase of 'fall' fish.

It has been suggested (Houston et al., 1968) that the muscle hydration associated with cold acclimation might be due to increased water permeability at the branchial level. If this were so, one would expect variations in the plasma electrolyte and water content as well. Since there was, in general, no change in these plasma parameters, it is unlikely that increased branchial water permeability at cold temperatures or, for that matter, decreased permeability with high temperatures occurs.

On the other hand, the osmotic concentration of blood is known to increase at lowered temperatures (Prosser, 1973). For instance, Houston and Fenwick (1964) have shown increases in total plasma protein of goldfish at low temperatures. This could cause a reduction in glomerular filtration, and if water entering the fish is not cleared fast enough, increases in tissue water might be expected.

Increased salt loss via the gills and kidney is another common problem faced by freshwater fish when exposed to higher environmental temperatures. However, acclimation to warm temperature (18°C) was accompanied by increased muscular ion concentrations in both series of fish. A number of possibilities could account for this.

It is possible that the ion uptake mechanisms, discussed in the Literature Review and in the plasma discussion (Fig. 7), overcompensate for this. This might result in some ion accumulation; i.e., ion uptake mechanisms are favored over active or passive ion extrusion mechanisms. The observed concentrations may have resulted from a concentrating effect due to apparent water loss at the higher temperatures compared to low temperatures. However, this is not likely, since in several cases where tissue water increased with temperature there was either no change or an increase in electrolyte concentrations. It is more likely that the observed variations developed from the

coordinated effects of other processes, such as the numerous metabolic pathways and the increased muscular activity in fish exposed to elevated temperatures.

Also of interest is the relative constancy of electrolyte distribution between the cellular and extracellular compartments (Tables 13 and 14). Although some variation was apparent, it is probably due to the generality of the approach. There was some indication of cellular Na^+ acclumulation with warm acclimation. Nevertheless, in general, despite temperature-related concentration changes, distribution of electrolytes between the two phases seemed to be maintained.

This distribution is important in excitatory tissues such as muscles and nerve, since the propagation of nervous stimuli and excitation-contraction coupling are affected by any alterations. The stability of the membrane potentials, or Nernst equilibrium potentials, presented in Figures 8, 9 and 10 j to 1, may indicate that these animals are compensating for temperature changes in such a way that stimulus-response relationships would be unaffected by temperature. The increase in E_K and E_{Cl} observed in the 'fall' series muscle samples was a result of increased plasma K^+ at 18°C. Correlation analyses indicated that plasma K was highly significantly correlated with E_K (see Appendix VI), which is consistent with observations that E_K is dependent upon external K^+.

The corresponding increase in E_{Cl} is related to the assumption of a Donnan distribution for K^+ and Cl^-. This assumed relationship ($Cl^-_i/Cl^-_e = K^+_e/K^+_i$) predicts that intracellular Cl^- will depend mainly on plasma K^+ concentrations (Cotlove and Hogben, 1962). This was supported by correlation analyses showing that plasma K^+ correlated very significantly with cellular Cl^- levels in muscle (Appendix VI).

As indicated earlier, the physiological significance, if any, of this increased plasma K^+ in 'fall' trout is unclear as is that of its effect on E_K.

7. Summary

1. In general, water content of 'summer' skeletal muscle did not vary with temperature, while that of 'fall-winter' tended to decrease with temperature. ECPV in both groups was generally smallest at the cold acclimation temperature (2°C). CPV of 'fall' animals was largest at 2°C, while that of 'summer' fish did not vary significantly with temperature.

2. Tissue electrolyte levels tended to increase with temperature, most significantly in 'fall' animals.

3. Cellular electrolytes of 'summer' skeletal muscle remained relatively stable over the temperature range employed. Cellular electrolyte levels in 'fall' skeletal muscle tended to increase with temperature.

4. Equilibrium potentials determined for skeletal muscle did not vary significantly with temperature in 'summer' animals. E_{Na} of 'fall' fish generally remained stable, while both E_{Cl} and E_K became less negative at 18°C.

5. Seasonal differences were apparent. The muscle of 'summer' fish contained more water and also had a larger cellular phase than that of 'fall' animals. 'Fall' muscle potassium levels were much larger than those of 'summer' animals. ECPV, sodium and chloride values were generally similar in both seasonal groups.

6. The distribution of electrolytes between the cellular and extracellular phase did not appear to vary with temperature. Approximately 50 to 60% of tissue sodium and 80 to 90% of tissue chloride was confined to the extra-

cellular compartment of skeletal muscle, while 99.9% of tissue potassium was located in the cellular compartment.

(C) Cardiac Muscle

1. Water Content and Distribution

Water content and distribution data are summarized in Table 8 ('summer') and Table 9 ('fall') and graphically presented in Figure 11 a to c.

'Summer Series'

It is of interest to note that the water content of cardiac muscle was the highest of the tissues tested. Water content did not, however, vary significantly over the temperature range (Table 8, Fig. 11a).

ECPV, as calculated from the chloride space, increased significantly ($P < 0.01$) at 18°C over values at 10°C (Fig. 11a), but was not significantly different from those at 2°C. The cellular phase volume decreased 18°C, although this was not significant (Fig. 11b).

'Fall-Winter' Series

In the 'fall' series fish water content was stable over the temperatures used (Fig. 11a, Table 10). ECPV, however, again increased significantly ($P < 0.01$) between 10° and 18°C, and 2° and 18°C (Fig. 11c), while cellular phase volume decreased significantly ($P < 0.01$) at 18°C (Fig. 11b).

Seasonal Differences

The tissue water content of the 'summer' series animals was higher at the two extreme temperatures than that of the 'fall' fish, although only by about 2%. In 'summer' fish, ECPV was substantially larger than was the case in cardiac muscle of 'fall' trout; in this instance by about 40% at any given acclimation temperature. By contrast, the cellular phase volume of 'fall' heart muscle was greater by approximately 13% than that of 'summer' fish at

Figure 11 Water and electrolyte parameters for cardiac muscle of rainbow trout acclimated to 2°, 10° and 18°C.

11a Water content

11b Cellular phase volume

11c Extracellular phase volume (Cl^- space)

11d Tissue sodium concentrations

11e Tissue chloride concentrations

11f Tissue potassium concentrations

11g Cell sodium concentrations

11h Cell chloride concentrations

11i Cell potassium concentrations

11j Sodium equilibrium potential (E_{Na})

11k Chloride equilibrium potential (E_{Cl})

111 Potassium equilibrium potential (E_K)

Horizontal line represents the mean; vertical bar is the 95% confidence interval of the mean. Light bar--'summer' series fish; dark bar -'fall-winter'series trout.

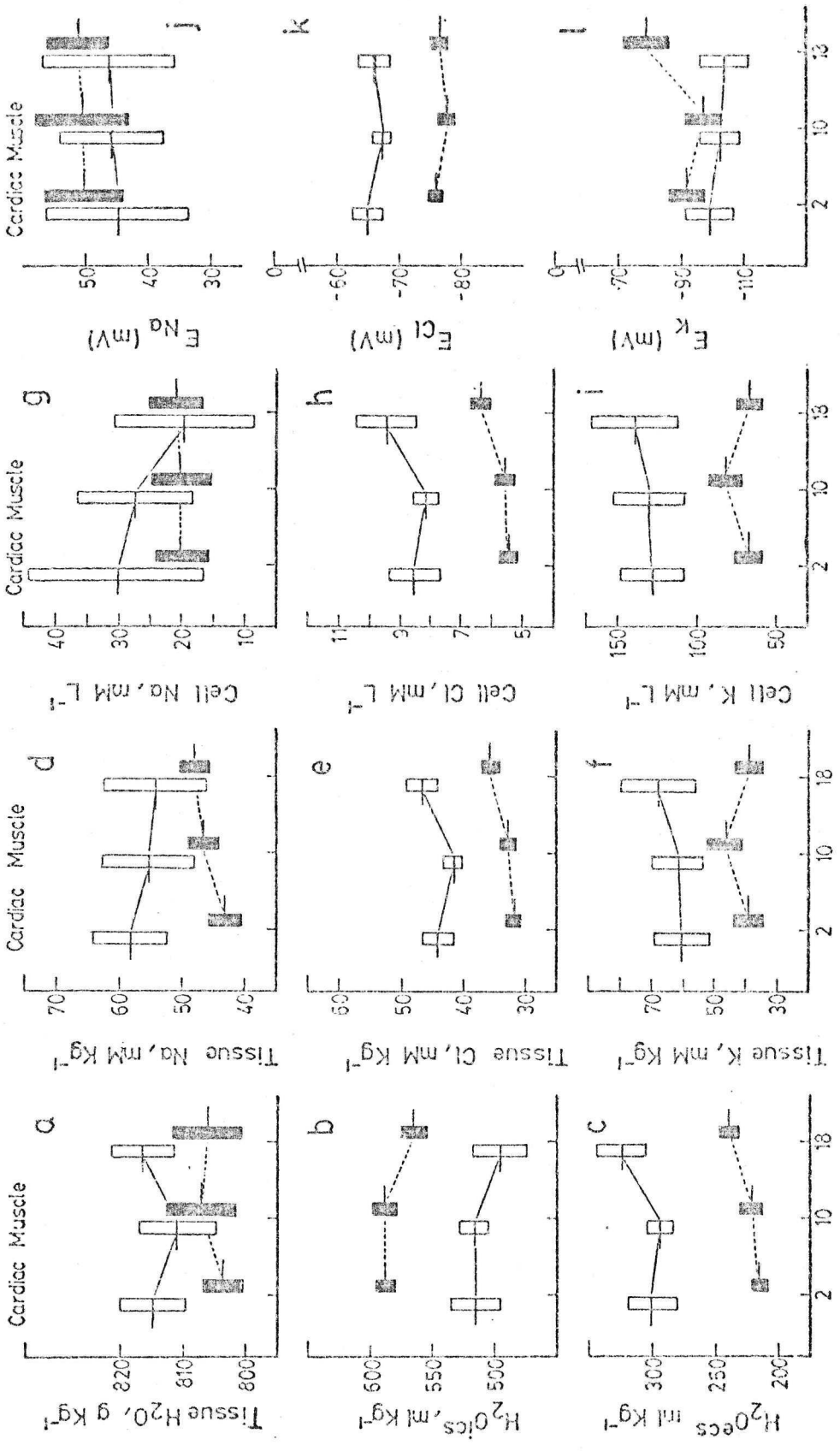

Cardiac Muscle
a
b
c
d
e
f
g
h
i
j
k
l
Tissue H2O, g kg-1
H2Oics, ml kg-1
H2Oecs ml kg-1
Tissue Na, mM kg-1
Tissue Cl, mM kg-1
Tissue K, mM kg-1
Cell Na, mM L-1
Cell Cl, mM L-1
Cell K, mM L-1
ENa (mV)
ECl (mV)
EK (mV)
TEMPERATURE, °C

Fig. 11

each acclimation temperature. The general pattern of response to increasing acclimation temperature was, however, similar in both groups.

2. Tissue Electrolyte Levels

'Summer' Series

Sodium: Cardiac muscle sodium content (Fig. 11d, Table 7) did not vary with temperature. Compared to skeletal muscle, sodium content of cardiac muscle was higher by some 40 mM kg^{-1}.

Chloride: The chloride content increased significantly ($P < 0.01$) at 18°C over that at 10°C, but was not significantly different from values at 2°C (Fig. 11e, Table 7). As was the case with sodium, cardiac muscle chloride content was greater than that of skeletal muscle by about 35 mM kg^{-1} at each acclimation temperature.

Potassium: Like sodium, potassium content remained fairly stable with temperature, varying less than 2% over the temperature range employed (Fig. 11f). Unlike sodium and chloride, however, the potassium content of cardiac muscle was less than that of skeletal muscle by 50 to 60 mM kg^{-1}.

'Fall-Winter' Series

As was the case with skeletal muscle, the response during acclimation was somewhat different in 'fall-winter' than in 'summer' animals.

Sodium: The sodium content of cardiac muscle increased ($P < 0.01$) between 2° and 18°C (Fig. 11d, Table 9).

Chloride: Cardiac muscle chloride content also increased with temperature, being significantly ($P < 0.01$) higher at 18° than at 2° and 10°C (Fig. 11e).

Potassium: On the other hand, potassium exhibited maximum concentration at the intermediate temperature (10°C) and lower, but similar concentrations at the two extremes (2°, 18°C) (Fig. 11f, Table 9).

Seasonal Differences

Seasonal differences were also obvious in this tissue. Electrolyte levels in the 'summer' series fish were, on the average, about 25% higher than those of 'fall-winter' animals. Sodium levels did not differ between groups as much as chloride and potassium but significant differences occurred between 2° and 10°C.

Response during acclimation also differed. 'Fall' sodium content increased with temperature, while 'summer' levels remained relatively stable. Potassium concentrations increased at 10°C over values at 2° and 18°C in 'fall' fish, but did not vary in 'summer' animals. 'Summer' chloride content did not change significantly between 2° and 18°C, while that of 'fall' cardiac muscle did.

3. Cellular Electrolyte Levels

'Summer' Series

Sodium: Cellular concentrations of sodium, like overall tissue sodium content did not vary significantly over the temperature range used (Fig. 11g, Table 11).

Chloride: Theoretically, the chloride space should not be used to calculate cell chloride levels, because of the underlying assumption. However, it has been used in the past (Ahokas and Duerr, 1975) and has been used in this study to determine whether cell chloride may change with temperature.

Cell chloride, like tissue chloride, increased between 10° and 18°C, but did not differ from values at 2°C (Fig. 11h, Table 11).

Potassium: As was the case with tissue levels, cellular potassium did not vary significantly with temperature.

'Fall-Winter' Series

Sodium: Unlike overall tissue Na^+ content, cell Na^+ levels remained relatively constant over the temperature range used (Fig. 11g, Table 12).

Chloride: Cell Cl^- concentrations followed the pattern seen in tissue levels of this electrolyte, increasing significantly ($P < 0.01$) at 18°C over values at 2° and 10°C (Fig. 11h).

Potassium: Cell K^+ concentrations also followed the patterns seen in overall tissue levels. Maximum K^+ concentration occurred at 10°C, while lower, but similar values were found at 2° and 18°C (Fig. 11i).

Seasonal Differences

As was the case with tissue levels of potassium and chloride, cell levels of these two electrolytes in 'summer' fish were higher than those of 'fall' animals. No seasonal variation was, however, observed in cell sodium concentrations. As previsouly noted, the response of cellular electrolytes, with the exception of sodium, in 'fall' animals, was similar to the pattern observed for overall tissue levels of these electrolytes.

4. Effect of Temperature on Electrolyte Distribution

The distribution of electrolytes between the cellular and extracellular compartments was also determined for cardiac muscle and is summarized in Tables 13 and 14.

Despite the similarities in actual tissue sodium, potassium and chloride concentrations, the inequality of distribution between the two phases was maintained. The extracellular sodium of cardiac muscle comprised approximately 75% of the total, with the remaining 25% confined to the cellular compartment. This is slightly different than the case of skeletal muscle where, on the average, 50 to 60 % of the sodium was extracellular. This difference presumably reflects the larger ECPV of cardiac muscle. Again, it

is difficult to ascertain whether the variations seen with temperature in distribution in 'summer' cardiac muscle are statistically significant, as calculations were carried out using mean values (Table 13).

Chloride distribution appeared very stable, in both 'fall' and 'summer' series animals, with 90% being extracellular and 10% cellular. There was no apparent variation with temperature.

Cellular potassium was approximately 99% of the total tissue content. The distribution of potassium in 'summer' cardiac muscle did not appear to vary with temperature (Table 13). However, in 'fall' animals, the precentage of potassium in the extracellular phase increased from 0.8% to 2.0% (Table 14). This was probably a combined result of increased plasma potassium at this temperature and the larger ECPV of cardiac muscle, as compared to skeletal muscle.

5. Nernst Equilibrium Potentials

The equilibrium potentials for sodium, chloride and potassium in cardiac muscle are presented in Figure 11 j to l. Neither E_{Na} nor E_{Cl} varied with temperature. E_K of 'summer' cardiac muscle also remained stable, but E_K in 'fall' fish increased towards zero at 18°C. Presumably, this is associated with the increased plasma potassium seen at this temperature. Compared to skeletal muscle, E_{Na} in cardiac muscle was, on the average, 20 mV less. Much the same was true of E_{Cl} in cardiac muscle, which was about 45 to 50 mV less negative than that in skeletal muscle. Cardiac muscle E_K was also less negative than that in skeletal muscle, although only by about 10 mV.

Seasonal differences were also observed. E_{Cl} in'summer' animals was less negative (Fig. 11k) than that in 'fall' animals. E_K of 'fall' fish

held at 18°C was less negative than that of 'summer' fish (Fig. 11-1).

6. Discussion

The only available report on thermoacclimatory effects on cardiac muscle electrolytes appears to be that of Murphy and Houston (1977). In the present study, tissue sodium concentrations in 'summer' fish were almost twice (i.e., 55 to 60 mM kg^{-1}, as compared to 24 to 30 mM kg^{-1}) those reported by Murphy and Houston (1977) for 'summer-photoperiod' trout. Values for 'fall-winter' fish were about 10-15 mM kg^{-1} larger than those observed for 'winter-photoperiod' animals from that same study. However, increases in the sodium concentrations of 'fall-winter' animals with temperature were seen in both studies. Potassium values of 'summer' fish in the present investigation were similar to values reported by these authors, while 'fall' potassium levels were some 10 to 20 mM kg^{-1} smaller. Values for tissue chloride and ECPV, as well as cellular electrolyte concentrations for cardiac muscle were not reported by Murphy and Houston (1977).

Cardiac muscle, although structurally and functionally different from skeletal muscle (the main physiological differences being the ability of cardiac muscle to beat spontaneously and rhythmically and the lack of voluntary control of contraction), relies on maintenance of ionic ratios for its proper functioning. Although the tissue levels of sodium and chloride were higher, and potassium lower than in skeletal muscle, inequalities in distribution were maintained; i.e., potassium was accumulated in the cellular compartment, whereas sodium and chloride were predominantly extracellular electrolytes. The distribution of potassium and chloride between the cellular and extracellular phases was similar in both skeletal

and cardiac muscle (Tables 13 and 14). Sodium distribution differed, with skeletal muscle apparently having higher percentages of the total sodium in the intracellular phase. The lower proportion of sodium in the cellular compartment in cardiac muscle might account for the smaller sodium equilibrium potential (E_{Na}) seen in cardiac as compared to skeletal muscle.

Despite variations in tissue and plasma sodium concentrations, E_{Na} remained constant and similar in both seasonal groups (Fig. 11j). E_K also remained stable across the temperature range, with the exception of 'fall' 18°C animals (Fig. 11-1). The importance of this in terms of action (E_{Na}) and resting membrane potentials (E_K) will be obvious in terms of cardiac muscle excitability. These animals appear to have compensated for temperature change in such as way as to allow cardiac muscle cells to function independently of potential temperature effects upon ion distribution. This, of course, is not the same as saying that heart rate and rate of muscular contraction are temperature-independent. However, it does imply regularity of contraction regardless of rate, as opposed to erratic muscle function as in the case of hyper- or hypoexcitability. The decrease in negativity of E_K (Fig. 11-1) seen in 'fall' series cardiac muscle at 18°C can be correlated with the increase in external or plasma potassium concentrations. As noted previously, the physiological significance of this increased plasma potassium is uncertain. It is clear, however, that it has the potential effect of causing cardiac muscle to become hyperactive.

7. Summary

1. Water content of cardiac muscle in both 'summer' and 'fall-winter' trout remained relatively stable with temperature. ECPV of 'fall' animals increased

with temperature, while that of 'summer' fish increased only between 10° and 18°C. CPV of 'fall' fish decreased at 18°C, while that of 'summer' fish did not vary significantly.

2. Tissue electrolyte levels in 'summer' series cardiac muscle also remained relatively stable with temperature. Tissue sodium and chloride of 'fall' cardiac muscle tended to increase with temperature, while potassium exhibited maximum concentration at 10°C.

3. Cellular electrolyte levels tended to follow the pattern observed in overall tissue content of electrolytes with respect to thermal acclimation.

4. Equilibrium potentials for each ion remained stable with temperature, with the only exception being E_K at 18°C in 'fall-winter' series cardiac muscle.

5. Seasonal differences were also apparent, with 'summer' animals having generally higher levels of electrolytes (both overall tissue and cell) than those of 'fall-winter' fish.

6. Distribution of electrolytes between the cellular and extracellular compartments did not vary with acclimation temperature, or with season. Approximately 75% of tissue sodium and 90% of tissue chloride were maintained in the extracellular compartment, while 99% of tissue potassium was located cellularly.

(D) Liver:

1. Water content and distribution

Water content data for liver are summarized in Figure 12 a to c and Tables 8 and 10.

'Summer' Series

Total liver water content did not vary with temperature and was the lowest of all tissues tested. Extracellular phase volume, calculated in this instance as Na^+ space, reached maximum values at 10°, which were significantly different ($P < 0.01$) from those at 2° or 18°C (Fig. 12c). As expected cellular phase volume was at a minimum at 10°C (Fig. 12 b).

'Fall-Winter- Series

As in 'summer' fish, no significant variation was observed in liver water content. Cold acclimation, (2°C) was accompanied by a significant decrease in ECPV ($P < 0.01$), and as expected, cellular phase volume exhibited a significant increase (Fig. 12 b and c) at this temperature.

Seasonal Differences

Total liver water content in the 'summer' series of fish was some 10 to 15 g kg^{-1} above that of 'fall-winter' trout at comparable temperatures. Cellular and extracellular phase volumes were similar in both groups at 2° and 10°C. At 18°C, however, ECPV of 'fall' fish was higher than that of 'summer' fish. The converse was true of the cellular phase volume.

2. Overall Tissue Electrolyte Levels.

'Summer' Series

Electrolyte data are summarized in Figures 12 d to f, and Table 7.

Sodium: Sodium concentrations (Fig. 12d) displayed maximum values at 10°C, and differed significantly from those seen at 2° ($P < 0.01$) and 18°C ($P < 0.05$).

Figure 12 Water and electrolyte parameters for liver of rainbow trout acclimated to 2°, 10° and 18°C

12a Water content

12b Cellular phase volume

12c Extracellular phase volume (Na^+ space)

12d Tissue sodium concentrations

12e Tissue chloride concentrations

12f Tissue potassium concentrations

12g Cell chloride concentrations

12h Cell potassium concentrations

12i Chloride equilibrium potential (E_{Cl})

12j Potassium equilibrium potential (E_K)

Horizontal line represent the mean, while the vertical bar is the 95% confidence interval of the mean. Light bars--'summer' series animals; dark bars-'fall-winter' series fish.

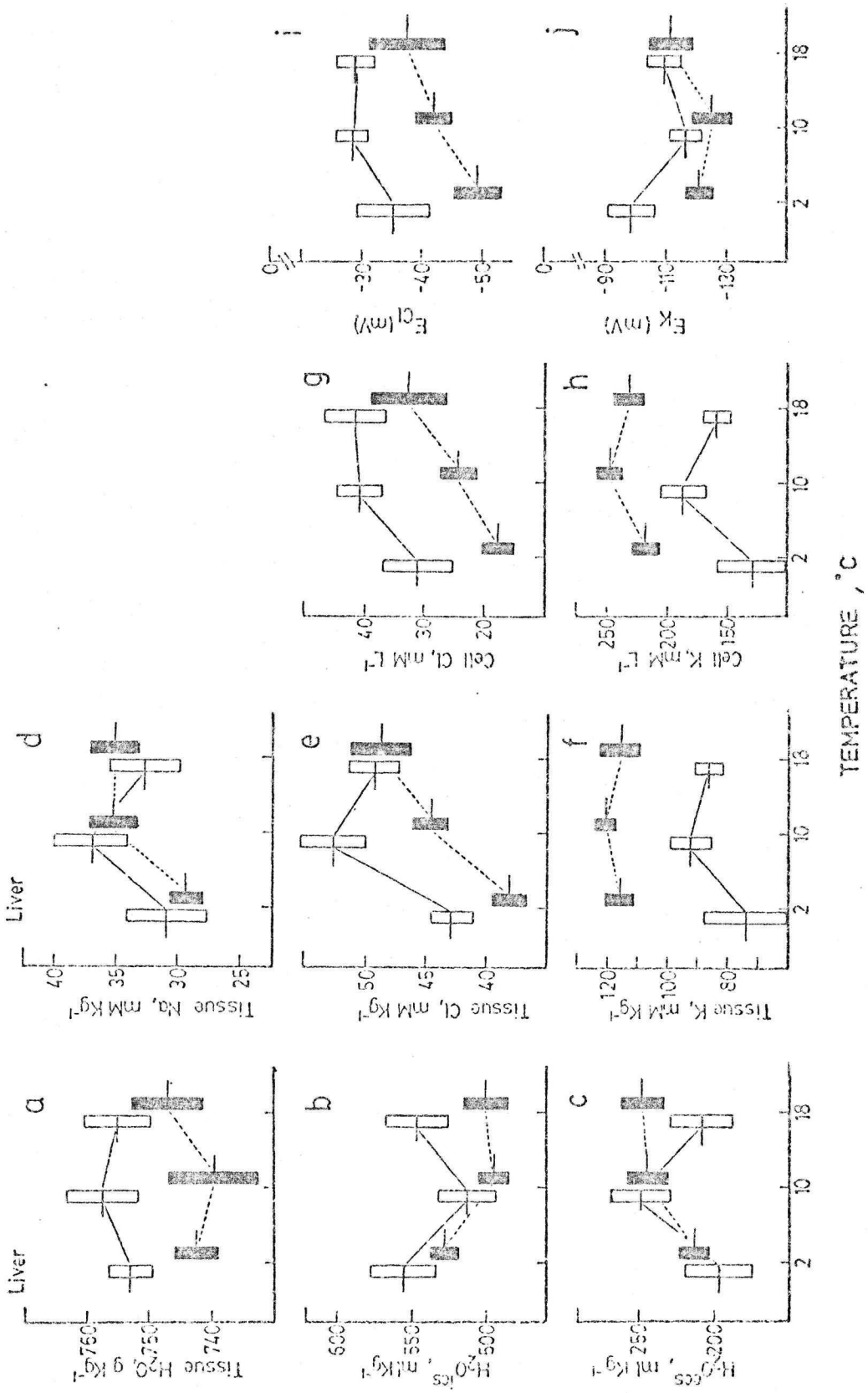

a
b
c
d
e
f
g
h
i
j
Liver
Liver
Tissue H2O, g kg-1
H2Oics, ml kg-1
H2Ocss, ml kg-1
Tissue Na, mM kg-1
Tissue Cl, mM kg-1
Tissue K, mM kg-1
Cell Cl, mM l-1
Cell K, mM l-1
ECl (mV)
EK (mV)
TEMPERATURE, °C

Fig. 12

Chloride: Tissue chloride varied in a similar fashion, with maximum values at 10°C (Fig. 12e). However, the concentration seen at 18°C was significantly ($P < 0.01$) in excess of that in cold-acclimated animals.

Potassium: Potassium increased between 2° and 18° ($P < 0.05$) but did not differ significantly between 10° and 18°C (Fig. 12f).

'Fall-Winter' Series

Electrolyte data are summarized in Figure 12 d to f and Table 9.

Sodium: In 'fall'animals' liver, sodium concentrations decreased with cold acclimation ($P < 0.01$), but were stable between 10° and 18°C (Fig. 12d).

Chloride: Tissue chloride levels increased significantly ($P < 0.01$) over each temperature interval tested (Fig. 12e).

Potassium: Potassium levels remained essentially constant over the temperature range tested (Fig. 12f).

Seasonal Differences

Variations between the 'fall-winter' and 'summer' groups were again apparent. In 'fall-winter' animals, potassium levels were, on the average, some 30 mM kg^{-1} higher than those of 'summer' animals. The potassium concentrations of 'fall' trout did not vary with temperature. Those of 'summer' animals, on the other hand, increased with temperature. 'Summer' fish chloride concentrations were higher than those of 'fall' trout, although the difference seen at 18°C was not significant. Sodium did not exhibit any significant seasonal differences.

3. Cellular Electrolytes

'Summer' Series

Intracellular electrolyte concentrations were calculated using sodium space estimates of ECPV. All calculations of liver cellular sodium yielded negative results for both 'summer' and 'fall' fish, regardless of the space

values used.

Chloride: Cell chloride was reduced at 2°C ($P < 0.05$), but remained stable between 10° and 18°C (Fig. 12g, Table 11).

Potassium: Cell potassium exhibited maximum concentration at 10°C; values at 2° and 18°C also differed significantly ($P < 0.05$) (Fig. 12h).

'Fall-Winter' Series:

Chloride: Cell chloride in 'fall' trout liver increased significantly ($P < 0.01$) with temperature, following the pattern seen with tissue levels of this electrolyte (Table 12).

Potassium: Cell potassium was also significantly increased ($P < 0.01$), but only between 2° and 10°C (Fig. 12h).

Seasonal Differences

The seasonal differences seen in the cellular potassium and chloride levels were comparable to those noted with respect to tissue levels of these electrolytes.

4. Effect of Temperature on Electrolyte Distribution

Following the procedure used with the other tissues, the distribution of electrolytes between the cellular and extracellular phases was determined. In 'summer' fish extracellular phase chloride accounted for 60% of total liver chloride at 2° and 10°C, and dropped to 54% at 18°C. Cellular phase chloride made up the difference, with 40% found at 2° and 10°, and 46% at 18°C (Table 13).

In contrast, the extracellular phase chloride of 'fall' trout liver accounted for 67 to 75% of the total (Table 14).

Cellular potassium made up 99.5% of total liver potassium in the'summer' fish and did not vary with temperature (Table 13). In 'fall' fish, 99.7% of

the potassium was in the cellular compartment. This value dropped very slightly to 99.3% at 18°C.

Thus, although liver appears to be highly thermosensitive with respect to electrolyte concentrations, distributions between cellular and extracellular phases remained relatively constant.

5. Nernst Equilibrium Potentials

Nernst equilibrium potentials calculated for chloride and potassium are displayed in Figure 12 i and j.

E_{Cl} of 'summer' animals increased in negativity at 2°C, but remained stable at 10° and 18°C (Fig. 12i). E_K, on the other hand, decreased in negativity at 2°C. In 'fall' trout variations in liver E_{Cl} were similar to those of the 'summer fish. In the 'fall' fish, E_K, in contrast to the 'summer' trout situation, decreased in negativity at 18°C (Fig. 12j). With respect to seasonal effects, it should be noted that 'summer' E_{Cl} and E_K values at 2°C were less negative than those of 'fall' animals.

6. Discussion

The results obtained in the present investigation were generally comparable to those obtained by Murphy and Houston (1977) for rainbow trout liver. There was one major difference, however, with respect to cellular chloride levels. Values encountered in this study were much higher (20 to 40 mM l^{-1}) than those of the above authors. This may have been the result of differences in the'space' used to estimate ECPV. As noted previously, sodium space was utilized in the present study, whereas Murphy and Houston (1977) used the chloride/potassium space. The chloride/potassium space

yielded larger ECPV estimates than those obtained by use of the sodium space in the present study. The use of these larger ECPV estimates would lead to smaller estimates of cellular chloride, but would not have much effect on the predominantly cellular electrolyte, potassium.

In comparison with values reported for perch liver by Lutz (1972), electrolyte levels seen in this study were lower (by about 4 mM kg^{-1} for sodium, and 5 to 29 mM kg^{-1} for potassium), although sodium spaces were comparable (267.3, for perch liver and 200 to 250 ml kg^{-1} for trout liver).

The liver plays a major role in affecting many aspects of cellular metabolism and a variety of homeostatic mechanisms. Almost all reactions of intermediary metabolism can take place in the liver. The fact that the liver does play a large role in metabolism may account for the large degree of apparent thermal sensitivity, since the control of metabolism in poikilotherms is very sensitive to temperature change (Behrisch, 1972). For example, the enzymes concerned with specific pathways of intermediary metabolism and energy production (e.g., the enzymes of glycolysis, gluconeogenesis, citric acid cycle, Na^+/K^+-ATPase and protein synthetic enzymes) undergo compensation during the acclimation process (Hazel and Prosser, 1974; Shaklee et al., 1977). The nature of this is generally such that the enzymes of cold-acclimated animals exhibit greater activity than those of warm-acclimated animals, at the same temperature. The observed modifications in trout liver water-electrolyte balance may have some effect on the intermediary metabolism. For example, Behrisch (1972) has shown that sodium and potassium can selectively inhibit or stimulate the activities of several glycolytic enzymes in marine poikilotherms in a manner determined by substrate and cofactor availability, as well as temperature. It has been suggested that

modifications in cation concentration may well be a method of controlling enzyme and metabolic activity (Bygrave, 1967; Behrisch, 1972).

A puzzling feature of liver ionic composition is the apparent lack of cellular sodium. This may have been an artefact of the type of calculations used in estimation of cellular sodium. However, all of the ion-defined space estimates of ECPV produced similar results. It is of interest to note that in the space evaluation study, similar results were obtained; the use of PEG-4000 and the ion-defined spaces gave negative results for cellular sodium. If there is any sodium present it must be at very low concentration. A possible explanation may be as follows. One of the functions of the liver is to secrete bile, which then enters the gall bladder. Vertebrate gall bladder bile is known to be an isotonic sodium-bile acid solution (Diamond, 1962, cited in Spence et al., 1977). If sodium is being secreted concomitantly with bile acids it could possibly account for the lack of cellular sodium in the liver.

Liver also had substantial amounts of cellular chloride present. In 'summer' animals liver cellular chloride levels were higher than in any other tissues, and in 'fall' fish second only to those of spleen. Manery (1954) attributes high liver cellular chloride content (about 50% of the total tissue chloride content) to blood cells trapped with the sinusoids, and the very large number of blood vessels in the liver. The liver has high blood content in trout (Stevens, 1968) and also receives a substantial percentage of the total blood flow (Cameron, 1976). Lutz,(1972) has suggested that the lack of chloride in secreted bile may be indicative of a chloride uptake mechanism.

7. Summary

1. Total water content did not vary with temperature, and was the lowest of all the tissues. ECPV of 'summer' animals was of maximum value at 10°C, while cellular phase volume was at a minimum at 10°C. In 'fall' fish, ECPV declined with cold acclimation, while cellular phase volume rose.

2. Tissue sodium and chloride of 'summer' fish displayed maximum concentrations at 10°C; potassium increased at 10°C over values at 2°C. Sodium and chloride of 'fall' trout liver tended to increase with temperature, while potassium remained relatively stable.

3. Cellular chloride concentrations generally increased with temperature in both groups, as did cellular potassium. Estimates of cellular sodium gave negative results.

4. E_{Cl} of 'fall' and 'summer' trout liver increased in negativity at 2°C. 'Summer' E_K decreased in negativity at 2°C, while that of 'fall' animals decreased at 18°C.

5. Despite variations in electrolyte levels with temperature, the distribution between cellular and extracellular phases remained relatively constant.

6. Seasonal variations existed in water content and electrolyte levels. 'Summer' water content was higher than that of 'fall' liver, as was 'summer' tissue and cellular chloride. In contrast tissue and cellular potassium levels of 'fall' trout were lower than those of 'summer' animals.

(E) Spleen

As noted earlier spleen was sampled only in 'fall' fish. Water-electrolyte data are summarized in Tables 9 and 10, and graphically displayed in Figure 13.

1. Water Content and Distribution

In contrast to the situation seen in other tissues, total water content increased ($P < 0.01$) at each of the acclimation temperatures (Fig. 13a). Extracellular phase volume, as estimated using the sodium space, was significantly ($P < 0.05$) higher at 18°C, than at the lower temperature (Fig. 13c). Cellular phase volume increased significantly ($P < 0.01$) between 2° and 10°C, but did not change thereafter (Fig. 13b).

2. Tissue Electrolyte Levels

Sodium: Warm acclimation was accompanied by a significant elevation ($P < 0.05$) in tissue sodium concentration (Fig. 13d, Table 9). Levels at 2° and 10°C did not differ significantly.

Chloride: Tissue chloride concentration displayed substantial and significant increases ($P < 0.01$) with temperature (Fig. 13c).

Potassium: Potassium concentrations on the other hand, were not significantly affected by acclimation (Fig. 13f).

3. Cellular Electrolyte Levels

Cellular electrolyte concentrations were calculated using the sodium space estimate of ECPV, and are summarized in Table 12.

Sodium: As was the case with liver, estimates of cellular sodium lead to negative values.

Figure 13. Water and electrolyte parameters for spleen of rainbow trout acclimated to 2°, 10° and 18°C.

13a Water content

13b Cellular phase volume

13c Extracellular phase volume (Na^+ space)

13d Tissue sodium concentrations

13e Tissue chloride concentrations

13f Tissue potassium concentrations

13g Cell chloride concentrations

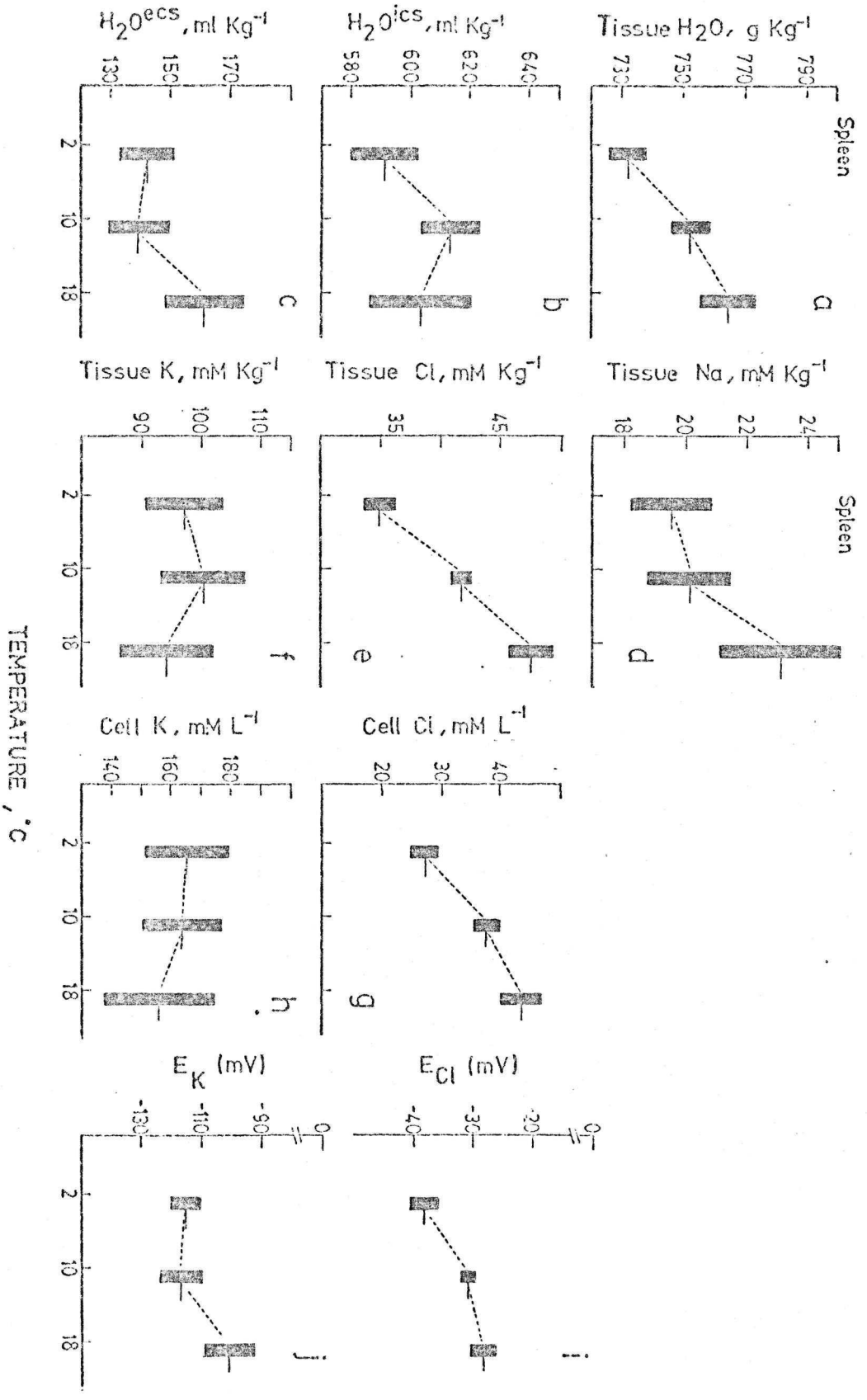
13h Cell potassium concentrations

13i Chloride equilibrium potential (E_{Cl})

13j Potassium equilibrium potential (E_K)

Horizontal line represents the mean; vertical bar is the 95% confidence interval of the mean. Dark bar--'fall-winter' series of fish; light bar--'summer' series of fish.

Fig. 13


Tissue H2O, g Kg-1
H2Oics, ml Kg-1
H2Oecs, ml Kg-1
Spleen
Tissue Na, mM Kg-1
Tissue Cl, mM Kg-1
Tissue K, mM Kg-1
Spleen
Cell Cl, mM L-1
Cell K, mM L-1
ECl (mV)
EK (mV)
TEMPERATURE, °C

Chloride: Cellular chloride concentrations increased significantly ($P < 0.01$) with temperature (Fig. 13g).

Potassium: Cellular potassium concentrations, like the tissue levels, remained essentially constant over the temperature range employed (Fig. 13h).

4. Effect of Temperature on Electrolyte Distribution

As before, distributions of chloride and potassium between the cellular and extracellular compartments were determined. Chloride appeared to be almost equally distributed between the two phases, ranging from 54 to 45% in the extracellular and from 46 to 55% in the cellular phase (Table 14). Although the amount of chloride in the extracellular phase dropped from 54% at 2° to 45% at 10° and 18°C, it was difficult to ascertain whether this was significant, for reasons stated previously. As in the other tissues, potassium remained constant in distribution with temperature; virtually all (≃99.8%) of the potassium being in the cellular phase.

5. Nernst Equilibrium Potentials

Nernst equilibrium potentials for chloride and potassium are shown in Figures 13 i and j. E_{Cl} became more negative at 2°C as compared to values at 10° and 18°C. Values for E_K became less negative at 18°C, compared to those at 2° and 10°C.

6. Discussion

There are no available reports on fishes with which to compare the results obtained in this study for spleen. However, potassium and chloride values are consistent with those cited by Manery (1954) for dog spleen.

Tissue levels of both sodium and chloride increased with temperature, while potassium did not vary. Tissue water increased with temperature, more so than any of the other tissues. Cellular chloride also rose significantly with temperature. The reason for this substantial increase and the lack of cellular sodium is not clear. The high chloride levels may be attributable to the large amount of blood and lymph tissue within the spleen. Stevens (1968) has reported high blood volumes in the spleen of the rainbow trout and suggests that it may serve a blood reservoir function. The increase in chloride levels and perhaps tissue water, could possibly be the result of increased bloodflow through the spleen at higher temperatures. Due to the lack of information, however, these explanations must be regarded as purely speculative.

7. Summary

1. Water content increased with temperature. ECPV was significantly higher at 18°C, and cellular phase volume was increased at 10°C compared to values at 2°C, but did not change thereafter.

2. Tissue levels of sodium and chloride increased with temperature. Potassium levels remained stable.

3. Cellular chloride concentrations increased with temperature, while potassium concentrations did not vary. Estimates of cellular sodium yielded negative results.

4. E_{Cl} increased in negativity at 2°C, while E_K decreased in negativity at 18°C.

5. Temperature had little effect on the distribution of electrolytes between the cellular and extracellular compartments.

(F) Gut

1. Water Content and Distribution

Water content data are summarized in Figures 14 a to c, and Tables 8 and 10.

'Summer' Series

Tissue water content did not vary significantly with acclimation temperature (Fig. 14a), nor did ECPV, as estimated by the chloride space (Fig. 14c). Cellular phase volume declined significantly ($P < 0.05$) between 2° and 10°C, but remained steady thereafter (Fig. 14b).

'Fall-Winter' Series

Tissue water content was not significantly affected by acclimation, although water distribution was altered. ECPV decreased ($P < 0.01$) with cold acclimation, while cellular phase volume increased under the same conditions.

Seasonal Differences

Water content of 'summer' animals was slightly greater (≃2-4%) than that of the 'fall' group. 'Fall' ECPV was, however, approximately 50% larger than the ECPV of 'summer' animals. In contrast, the 'summer' cellular phase volume was almost 40% larger than that of the 'fall' trout gut.

2. Tissue Electrolytes

Tissue electrolyte data are summarized in Figures 14 d to f, and Tables 7 and 9.

'Summer' Series

The gut tissue of 'summer' trout was noteworthy for the absence of any significant variation in tissue sodium, potassium and chloride content with acclimation (Table 7).

Figure 14 Water and electrolyte parameters for gut tissue of rainbow trout acclimated to 2°, 10° and 18°C.

14a Water content

14b Cellular phase volume

14c Extracellular phase volume (Cl^- space)

14d Tissue sodium concentrations

14e Tissue chloride concentrations

14f Tissue potassium concentrations

14g Cell chloride concentrations

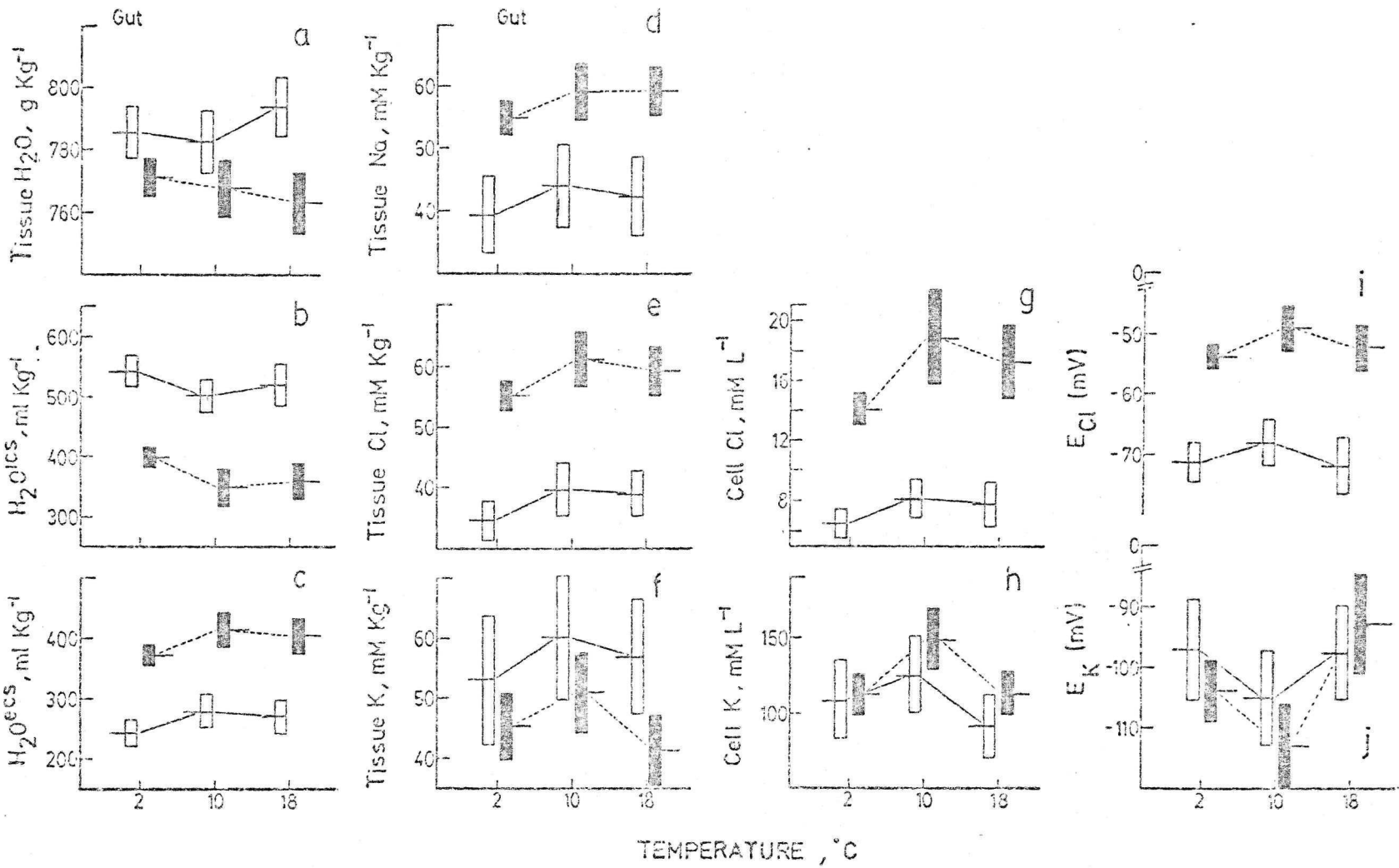
14h Cell potassium concentrations

14i Chloride equilibrium potential (E_{Cl})

14j Potassium equilibrium potential (E_K)

Horizontal line represents the mean; vertical bar is the 95% confidence interval of the mean. Light bars--'summer' series trout; dark bars--'fall-winter' series fish.

Fig·14


Gut
a
Tissue H_2O, g Kg^{-1}
800
780
760
b
H_2O^{ics}, ml Kg^{-1}
600
500
400
300
c
H_2O^{ecs}, ml Kg^{-1}
400
300
200
Gut
d
Tissue Na, mM Kg^{-1}
60
50
40
e
Tissue Cl, mM Kg^{-1}
60
50
40
f
Tissue K, mM Kg^{-1}
60
50
40
g
Cell Cl, mM L^{-1}
20
16
12
8
h
Cell K, mM L^{-1}
150
100
i
E_{Cl} (mV)
0
-50
-60
-70
j
E_K (mV)
0
-90
-100
-110
2
10
18
TEMPERATURE , °C

'Fall-Winter' Series

Sodium: Sodium content of 'fall' animals, like that of 'summer' trout, did not change with acclimation (Fig. 14 d).

Potassium: Tissue potassium concentration declined ($P < 0.05$) between 10° and 18°C, although values at 2° and 18°C did not differ (Fig. 14 f).

Chloride: Chloride content rose significantly between 2° and 10° ($P < 0.05$) although that at 2° and 18°C, like potassium, did not differ (Fig. 14 e).

Seasonal Differences

Levels of gut tissue sodium and chloride in 'fall' fishes were some 15 to 20 mM kg^{-1} higher than those in 'summer' animals. Potassium differed between the two groups only at 18°C. In this case, the potassium concentrations in 'summer' fish were significantly higher ($P < 0.01$) than those in 'fall' trout. As noted above, electrolyte levels in 'summer' fish were generally unaffected by acclimation; a situation which contrasts with that observed in the 'fall' series of fish.

3. Cellular Electrolyte Levels

Cellular electrolyte concentrations were calculated using the chloride space estimate of ECPV, and are summarized in Tables 11 and 12. As in liver and spleen, estimation of cellular sodium levels lead to negative values.

'Summer' Series

Following the pattern seen in tissue levels, neither cellular chloride nor potassium concentrations varied significantly with acclimation temperature (Table 11).

'Fall-Winter' Series

Chloride: In the 'fall-winter' animals, cell chloride levels reached minimum levels ($P < 0.05$) with cold-acclimation (Fig. 14 g).

Potassium: Maximum cellular potassium concentrations were observed at 10°C ($P < 0.05$), while values at 2° and 18°C were similar (Fig. 14 h).

Seasonal Differences

Seasonal variation was observed only in the case of chloride. In 'fall' fish, chloride concentrations were some 10 mM l^{-1} higher than those of 'summer' animals.

4. Effect of Temperature on Electrolyte Distribution

Chloride in the extracellular phase accounted for 90% of the total present. This did not change with either temperature or season (Tables 13 and 14).

The distribution of potassium was similar to that seen in the other tissues with approximately 99% of potassium being in the cellular compartment. In 'fall' animals, however, potassium in the extracellular phase increased from 1% of the total at 2° to 3% at 18°C. 'Summer' trout remained relatively constant with respect to potassium distribution.

5. Nernst Equilibrium Potentials

Equilibrium potentials for chloride and potassium are displayed in Figure 14 i and j. In 'summer' animals, neither E_{Cl} or E_K varied significantly with temperature. On the other hand, potentials estimated for the 'fall' group did show some changes. For instance, E_{Cl} was significantly ($P < 0.05$) more negative at 2°, than at 10° or 18°C. E_K displayed maximum negativity at 10°C; values at 18°C were least negative (Fig. 14 j). Seasonal differences were observed only in the case of the chloride potentials where those of 'fall' specimens were some 15 to 20 mV less negative than the value obtained in 'summer' animals.

6. Discussion

Only Lutz (1972) has reported values for gut tissue electrolyte levels in fishes; in this instance for the perch. Although water contents were similar, electrolyte concentrations were much higher than those observed for trout gut. The chloride spaces obtained by Lutz (1972) for perch gut were also much larger than those for trout gut. This was true, as well, of estimated cellular sodium and chloride concentrations. Only in the case of cellular potassium concentrations were the values obtained by Lutz comparable to those noted in this study. On the other hand, Ward and Stokes (1969) have reported that sections of trout mid-gut has extracellular phase volumes which comprise about 25% of the wet tissue weight and these values are comparable to those in the 'summer' series of fish.

As was the case with liver and spleen, gut tissue appeared to lack cellular sodium. Although this may be an artefact of the calculations involved, any sodium present must be at very low concentrations.

In fish, the intestine participates in the transfer of amino acids and glucose across the intestinal wall; processes which are coupled with sodium uptake via a transport carrier (Hazel and Prosser, 1974). The sodium uptake is dependent upon the maintenance of low intracellular sodium concentration (Knoebel, 1971), and the sodium which enters the cell with the carrier is actively transported out by a sodium pump.

Cellular chloride, which was observed to be among the highest concentrations of the tissues tested, is thought to be a result of the large number of gland cells in the intestinal mucosa (Manery, 1959).

The lack of any great variation in electrolyte and water levels with acclimation may be explained, in part, by the fact that the gut of freshwater fishes, unlike that in marine fishes, plays a negligible role

in ion regulation. Since freshwater fish drink little water and can maintain salt balance during periods of starvation (Maetz, 1971), it would appear that the gut does not greatly participate in electrolyte recruitment from the medium or diet.

7. Summary

1. Tissue water content did not vary with temperature, nor did ECPV of 'summer' trout. 'Fall' ECPV decreased with cold acclimation, while cellular phase volume of both groups increased under the same condition.

2. Tissue electrolytes of 'summer' trout gut also lacked significant variation with temperature. 'Fall' tissue chloride levels increased between 2° and 10°C; potassium decreased between 10° and 18°C. 'Fall' sodium showed no variation.

3. Neither cellular chloride nor potassium of 'summer' animals changed with temperature. 'Fall' cellular chloride reached minimum levels with cold acclimation, while potassium concentrations were at maximum values at 10°C.

4. In 'summer' animals, neither E_{Cl} nor E_K varied with temperature. 'Fall' E_{Cl} was more negative at 2°C than at 10° or 18°C. E_K was of maximum negativity at 10°C and of minimum negativity at 18°C.

5. Distribution of electrolytes between the cellular and extracellular phases appeared to be unaffected by temperature.

6. Seasonal differences did exist. 'Fall' ECPV and electrolyte levels, with the exception of potassium were higher than those in 'summer' trout. 'Summer' cellular phase volumes were larger than those of the 'fall' fish.

(G) Brain

1. Water Content and Distribution

'Summer' Series

Water content was at a minimum at 10°C, and significantly ($P < 0.05$) below the value at 18°C, but not at 2°C (Table 8, Fig. 15a). No significant differences were apparent between the 2° and 18°C groups. ECPV, as estimated from the chloride space, also increased ($P < 0.05$) with warm acclimation (Fig. 15 c), while the converse was true of cellular phase volume (Fig. 15 b). It is interesting to note that the ECPV of brain was the highest of the tissues analyzed and correspondingly, the cellular phase volume was the smallest.

'Fall' Series

In contrast to the situation in 'summer' fish, brain water content in 'fall' trout increased ($P < 0.05$) at 10° over that at 2°C. Values at 2° and 18°C and 10° and 18°C did not differ significantly (Fig. 15 a, Table 10). In these animals, the brain water content was one of the highest of the tissues analyzed. Cold acclimation was associated with a significant ($P < 0.05$) decline in ECPV (Fig. 15 c), with the opposite being true of cellular phase volume. ECPV of 'fall' trout brain was not the largest of the tissues used, as was the case in 'summer' fish.

Seasonal Differences

Brain water content displayed seasonal variation only at 10°C, where levels in 'fall' fish were about 10 g kg^{-1} higher than those of 'summer' animals. Neither ECPV nor cellular phase volumes showed seasonal differences.

Figure 15. Water and electrolyte parameters for brain of rainbow trout acclimated to 2°, 10° and 18°C.

15a Water content

15b Cellular phase volume

15c Extracellular phase volume (Cl^- space)

15d Tissue sodium concentrations

15e Tissue chloride concentations

15f Tissue potassium concentrations

15g Cell sodium concentrations

15h Cell chloride concentrations

15i Cell potassium concentrations

15j Sodium equilibrium potential (E_{Na})

15k Chloride equilibrium potential (E_{Cl})

15l Potassium equilibrium potential (E_K)

Horizontal line represents the mean; vertical bar is 95% confidence interval of the mean. Light bars--'summer' series fish; dark bars--'fall-winter' series fish.

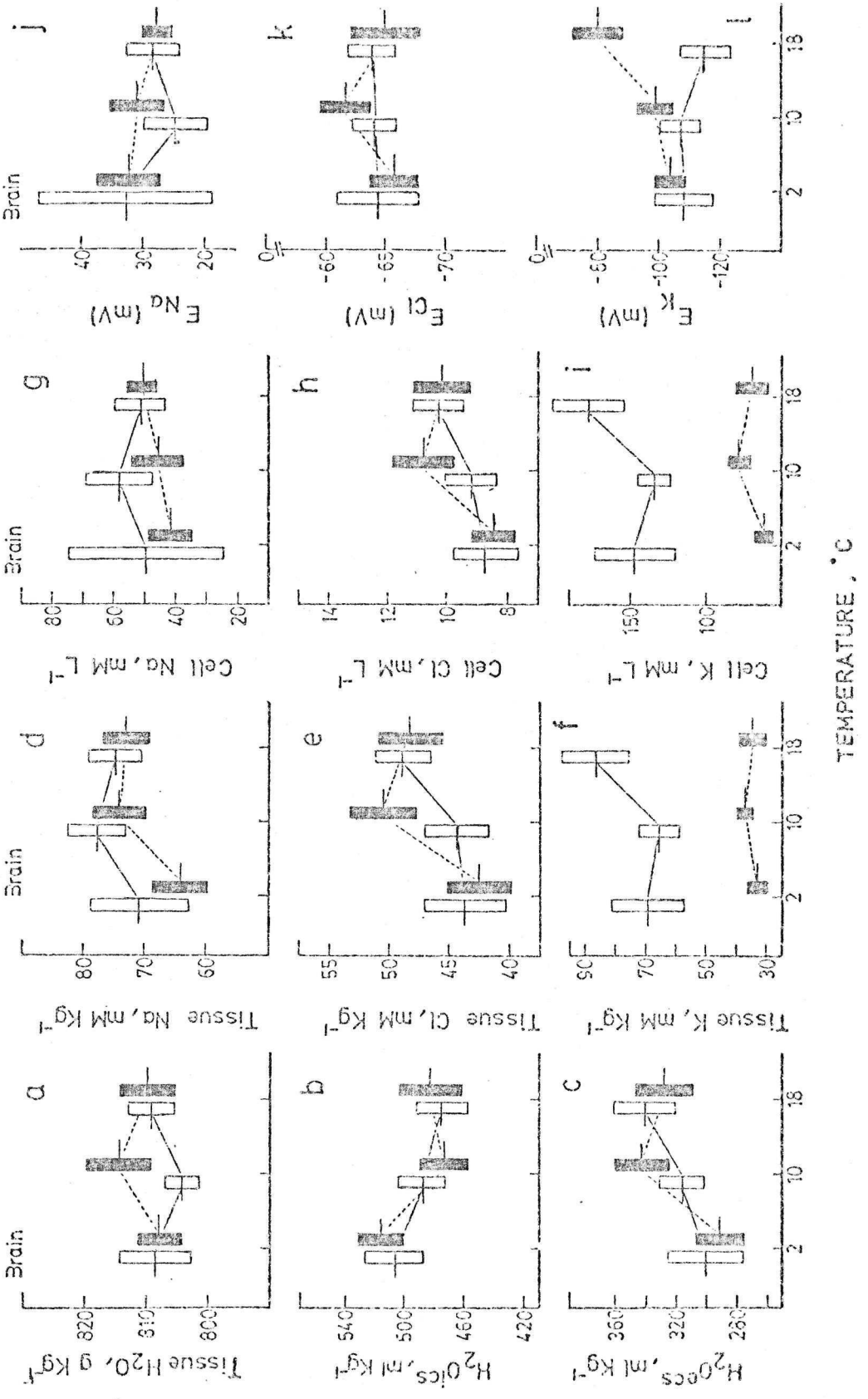

a
b
c
d
e
f
g
h
i
j
k
l
Brain
Tissue H2O, g kg-1
H2Oics, ml kg-1
H2Oecs, ml kg-1
Tissue Na, mM kg-1
Tissue Cl, mM kg-1
Tissue K, mM kg-1
Cell Na, mM L-1
Cell Cl, mM L-1
Cell K, mM L-1
ENa (mV)
ECl (mV)
EK (mV)
TEMPERATURE, °C

Fig.15

2. Tissue Electrolyte Levels

Tissue electrolyte data are summarized in Figures 15 d and e, and Tables 8 and 9.

'Summer' Series

Sodium: Sodium content of brain appeared to be unaffected by acclimation. Brain sodium levels were however the highest encountered in any of the tissues investigated.

Chloride: In contrast to sodium, chloride content increased ($P < 0.01$) with acclimation to 18°C (Fig. 15 e).

Potassium: Tissue potassium concentration also displayed significant ($P < 0.01$) elevation with acclimation to 18°C (Fig. 15 f).

'Fall-Winter' Series

Sodium: In 'fall-winter' animals, cold acclimation was accompanied by a decline ($P < 0.01$) in tissue sodium content (Fig. 15 d, Table 9).

Chloride: Tissue chloride levels displayed similar variations, decreasing ($P < 0.01$) at 2°C, compared to 10° and 18°C.

Potassium: Potassium concentrations were significantly elevated ($P < 0.05$) between 2° and 10°C. Values at 10° and 18°, and 2° and 18°C were not significantly different.

Seasonal Differences

Only potassium and chloride concentrations at 10°C exhibited seasonal variations. Levels in 'summer' trout brain were much higher (30-50 mM kg^{-1}) than those of fall animals. at 10°C, 'fall' chloride content was greater ($\simeq$5 mM kg^{-1}) than that of the 'summer' trout brain.

3. Cellular Electrolyte Levels

Cellular electrolyte concentrations were calculated using the chloride space estimate of ECPV, and are summarized in Figures 15 g to i, and Tables 11 and 12.

'Summer' Series

Sodium: Cell sodium concentrations displayed no significant variation with acclimation. Like tissue levels, brain cell sodium concentrations were the highest of any of the tissues tested.

Chloride: Cell chloride concentrations increased significantly ($P < 0.05$) with acclimation to 18°C (Fig. 15 h).

Potassium: Cellular concentration of potassium were significantly ($P < 0.01$) elevated at 18°C over values at 10°C, but did not differ from those at 2°C (Fig. 15 i, Table 11).

'Fall-Winter' Series

Sodium: Cell sodium levels of 'fall-winter' animals were higher at 18°C ($P < 0.05$) than at 2°C (Table 12).

Chloride: Cellular chloride concentration, like overall tissue levels declined ($P < 0.01$) with cold acclimation (Fig. 15 h).

Potassium: Similar to tissue levels, cell potassium rose significantly ($P < 0.01$) at 10°C when compared with concentrations at 2°C, but did not differ from values at 18°C (Fig. 15 i).

Seasonal Differences

Seasonal differences in cellular electrolyte levels were similar to those observed in overall tissue levels.

4. Effect of Temperature on Electrolyte Distribution

Distributions of sodium, potassium and chloride between the two phases are summarized in Tables 13 and 14.

Between 63 and 71% of brain sodium content was allocated to the extracellular phase, while the cellular phase contained between 29 and 37%. The variation observed cannot be considered significant, since the calculations involved only mean values. Moreover, there was no apparent difference in distribution with respect to either acclimation or season.

Extracellular chloride accounted for 90% of the total brain chloride in both 'summer' and 'fall' fish. The remaining 10% was confined to the cellular phase.

In 'summer' fish, 99% of the potassium was cellular; 'fall' fish had 97-98% in the cellular phase. Potassium in the extracellular phase of 'fall' animals increased from 1.3% at 2° to 3% at 18°C. Although this difference is small, it may be of some importance in brain function, as will be discussed below.

5. Nernst Equilibrium Potentials

Equilibrium potentials for sodium, potassium and chloride are presented in Figures 15 j to 1. E_{Na} did not vary with temperature in either the 'summer' or 'fall' animals. E_{Cl} and E_K of 'summer' trout brain also remained relatively stable over the temperature range used. In 'fall' fish E_{Cl} was less negative at 10° than at 2°C, but did not differ significantly from values seen in the 18°C group (Fig. 15 k). As was typically the case in other tissues, 'fall' fish exhibited significant decrease in E_K negativity at 18°C (Fig. 15 1). The only seasonal variation observed was that of E_K at 10°C, with 'fall' E_K being less negative than that of 'summer' fish.

6. Discussion

The results reported here for brain parameters are comparable with those of Hickman et al. (1964), with one exception. Whereas Hickman et al. (1964) found increased tissue chloride with cold acclimation, decreased chloride levels were observed in the present study.

As seen in Tables 13 and 14, the distribution of electrolytes between the cellular and extracellular compartment remained relatively constant with temperature. The only exception to this generalization was encountered in 18°C animals of the 'fall-winter' series. Here the increase in plasma potassium was associated with some increase in the proportion of total potassium allocated to the extracellular phase.

The vital importance of stabilizing extracellular potassium levels in relation to regulation of brain activity has been well documented (Katzman, 1976; Pedley et al., 1976). Small changes in extracellular potassium are normally observed with neuronal activity and probably play an important part in modulating local excitability (Pedley et al., 1976). It has been shown that large changes (increases of three to four times a baseline of three mEq l^{-1}) in extracellular potassium concentrations can be correlated with epileptic seizures in mammalian brains, and may be related to the changes in membrane potentials which also follow from variations in extracellular potassium.

With reductions in extracellular potassium concentrations, relative to those within brain cells, the potassium equilibrium potential (E_K) becomes greater (i.e., more negative). Katzman (1976) suggests that maintenance of low extracellular potassium provides a high E_K against which membrane potentials may be more easily maintained. As can be seen from Table 14 and Figure 15 1, the small increase (from 1.4% to 3.0% of the

total) in extracellular phase potassium lead to a rather large significant ($P < 0.01$) decrease in negativity ($\simeq$20 mV) of E_K. This may also lead to alterations in neural activity in the 'fall' 18°C group of trout, if the situation seen in mammalian brains is also true in the lower vertebrates. This, however, is only speculation.

7. Summary

1. 'Summer' water content increased between 10° and 18°C, but did not differ at 2° and 18°C. 'Fall' water exhibited maximum levels at 10°. 'Summer' ECPV increased with warm acclimation; converse was true of cellular phase volume. Cold acclimation was accompanied by a decline in 'fall' ECPV and a rise in cellular phase volume.

2. Brain sodium content of 'summer' trout did not vary with temperature, while chloride and potassium levels increased with warm acclimation. Sodium and chloride of 'fall' animals declined with cold acclimation; potassium levels were elevated at 10°C compared to 2°C, but did not differ from levels at 18°C.

3. Cellular electrolyte concentrations in 'summer' and 'fall' trout brain generally followed the pattern seen in tissue levels of these electrolytes.

4. E_{Na} did not vary with temperature in either group. E_{Cl} and E_K of 'summer' fish also remained relatively constant. E_{Cl} of 'fall' fish became less negative at 10°C, while the same was true of E_K at 18°C.

5. Electrolyte distribution did not appear to be altered by temperature in 'summer' animals, although potassium in the extracellular phase increased in 'fall' animals at 18°C.

6. Only potassium concentrations differed on a seasonal basis, with 'summer' levels being much higher than those of 'fall' animals.

(H) Summarizing Discussion

The data presented indicate that water-electrolyte balance in rainbow trout undergoes some change following thermal acclimation. Furthermore, there appear to be variations attributable to 'season'.

Appreciating that exposure of 'fall-winter' fish to 18°C, and 'summer' trout to 2°C represents a profound departure from normal seasonal circumstances, results obtained have been compared by season at each of the three temperature intervals (i.e., 2° to 10°, 10° to 18° and 2° to 18° C). This summary is presented in Table 15.

It is apparent from Table 15 that both temperature and seasonal differences exist. In addition, specific variations at the tissue level can be identified. For example, cardiac muscle of 'summer' fish, when compared at 2° and 10°C exhibits no change in any of the parameters tested. Between 10° and 18°C, the same tissue exhibits 25% variation, while when compared at the two extreme situations again shows no variation. In contrast, cardiac muscle of 'fall-winter' trout responds to acclimation with more variation, the minimum being 33% variation between 2° and 10°C.

It is also clear that some tissues are more thermosensitive than others. Liver of 'summer' fish appears to be particularly sensitive with respect to temperature, exhibiting an average 67% change in all comparisons. Brain was also sensitive with an average 31% variation overall in 'summer' trout.

Among 'fall-winter' animals, however, the situation was different. Although liver and brain were again thermosensitive, the tissue showing the most variation was spleen. Muscle samples also exhibited more variation with temperature than those in 'summer' animals. The similarity in response of the parameters in brain of 'summer' fish compared at 2° to 10° and 'fall' fish at 10° to 18°C was interesting. Both groups showed lack of significant change with temperature when compared at these intervals.

TABLE 15. Summary of results grouped according to season and temperature interval. I represents an increase in value, D indicates a decrease and NS represents no significant variation. The percent change out of the total possible change is also reported.

		Na_t	Na_c	Cl_t	Cl_c	K_t	K_c	H_2O	H_2O^{ecs}	H_2O^{ics}	E_{Na}	E_K	E_{Cl}	% CHANGE
SUMMER 2°-10°	Post Opercular	I	NS	I	NS	NS	NS	NS	I	D	NS	NS	NS	33
	Mid-Dorsal	NS	NS	I	NS	I	I	NS	I	NS	NS	NS	NS	33
	Caudal Muscle	NS	NS	NS	NS	NS	NS	I	NS	NS	NS	NS	NS	8
	Cardiac Muscle	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	Liver	I	--	I	I	I	I	NS	I	D	--	I	D **	90
	Gut	NS	--	NS	NS	NS	NS	NS	NS	D	--	NS	NS	7
	Brain	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	% CHANGE	29	0	43	14	29	27	14	43	43	0	14	14	
FALL 2°-10°	Post-Opercular	I	NS	I	NS	NS	NS	NS	I	D	NS	NS	NS	33
	Mid-Dorsal	I	I	I	NS	NS	I	NS	I	D	NS	NS	NS	50
	Caudal Muscle	I	NS	I	NS	NS	NS	NS	NS	NS	NS	NS	NS	17
	Cardiac Muscle	I	NS	NS	NS	I	I	NS	NS	NS	NS	NS	I	33
	Liver	I	--	I	I	NS	I	NS	I	D	--	NS	D	70
	Spleen	NS	--	I	I	NS	NS	I	NS	I	--	NS	D	50
	Gut	NS	--	I	I	NS	I	NS	I	D	--	I	D	70
	Brain	I	NS	I	I	I	I	I	I	D	NS	NS	D	75
	% CHANGE	75	20	87	50	25	62	25	62	75	0	12	62	
SUMMER 10°-18°	Post-Opercular	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	Mid-Dorsal	NS	NS	NS	I	NS	NS	NS	NS	NS	NS	NS	NS	8
	Caudal Muscle	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	Cardiac Muscle	NS	NS	I	I	NS	NS	NS	I	NS	NS	NS	NS	25
	Liver	D	--	D	NS	NS	D	NS	D	I	--	NS	NS	50
	Gut	NS	--	NS	NS	NS	NS	NS	NS	NS	--	NS	NS	0
	Brain	NS	NS	I	I	I	I	I	I	NS	NS	NS	NS	50
	% CHANGE	14	0	43	43	14	27	14	43	14	0	0	0	
FALL 10°-18°	Post-Opercular	NS	I	NS	I	NS	NS	NS	NS	NS	NS	D	D	33
	Mid-Dorsal	I	I	NS	I	NS	NS	NS	D	NS	D	D	D	58
	Caudal Muscle	NS	I	I	I	NS	NS	NS	NS	NS	NS	D	D	42
	Cardiac Muscle	NS	NS	I	I	D	D	NS	I	D	NS	D	NS	58
	Liver	NS	--	I	NS	NS	NS	NS	NS	NS	--	D	NS	20
	Spleen	I	--	I	I	NS	NS	I	I	NS	--	D	D	70
	Gut	NS	--	NS	NS	D	D	NS	NS	NS	--	D	NS	30
	Brain	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	D	I	17
	% CHANGE	25	60	50	62	25	25	12	37	12	20	100	62	

		Na_t	Na_c	Cl_t	Cl_c	K_t	K_c	H_2O	H_2O^{ecs}	H_2O^{ics}	E_{Na}	E_K	E_{Cl}	% CHANGE
SUMMER 2°-18°	Post-Opercular	I	NS	I	NS	NS	NS	NS	I	D	NS	NS	NS	33
	Mid-Dorsal	NS	NS	I	NS	NS	NS	NS	I	NS	NS	NS	NS	17
	Caudal Muscle	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	Cardiac Muscle	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
	Liver	NS	--	I	I	I	I	NS	NS	NS	--	I	D	60
	Gut	NS	--	NS	NS	NS	NS	NS	NS	NS	--	NS	NS	0
	Brain	NS	NS	I	I	I	NS	NS	I	D	NS	NS	NS	42
	% CHANGE	14	0	57	29	29	14	0	43	29	0	14	14	
FALL 2°-18°	Post-Opercular	I	I	I	I	NS	NS	D	NS	D	NS	D	D	67
	Mid-Dorsal	I	I	I	I	I	I	D	NS	NS	D	D	D	83
	Caudal Muscle	I	I	I	I	NS	NS	D	NS	NS	NS	D	D	58
	Cardiac Muscle	I	NS	I	I	NS	NS	NS	I	D	NS	D	NS	50
	Liver	I	--	I	I	NS	NS	NS	I	D	--	D	D	70
	Spleen	I	--	I	I	NS	NS	I	I	NS	--	D	D	70
	Gut	NS	--	NS	I	NS	NS	NS	NS	D	--	D	NS	30
	Brain	I	I	I	I	NS	NS	NS	I	D	NS	D	NS	58
	% CHANGE	87	80	87	100	12	50	50	50	2	20	100	62	

** in the case of E_{Na}, E_K and E_{Cl} D = a decrease towards zero, or a decrease in negativity, and I = an increase in negativity, for E_K and E_{Cl}.

Seasonal differences in response patterns are also clearly evident from this summary. Some tissues of 'summer' trout showed no significant alteration in any of the parameters analyzed, while in 'fall' fish no tissue exhibited less than 17% variation with temperature. It would appear that the 'summer' group of fish were more capable of maintaining tight control over their water-electrolyte balance than the 'fall' fish. The reason for this remains unclear.

Of all the parameters analyzed, sodium and chloride levels appeared to be the most thermolabile, and particularly so in 'fall-winter' animals. ECPV and cellular phase volume were also comparatively variable. Levels of the major cellular electrolyte, potassium, were relatively stable.

The tissues most affected by temperature were those primarily involved with

(i) intermediary metabolism, i.e., the liver and spleen, and

(ii) with control, i.e., the brain.

This would seem to support the hypothesis of metabolic reorganization during thermal acclimation (Hochachka and Somero, 1971). Tissues, such as skeletal muscle and gut did not appear to be affected as much, i.e., compensation seemed to be near-perfect. Cardiac muscle, the central feature of the circulatory system, also appeared to compensate well for temperature change.

Another point of importance concerns the effect of temperature upon electrolyte distribution. In spite of the changes that did occur, the evidence suggests that electrolyte distribution between the cellular and extracellular phases remained constant. In other words, although the absolute amounts varied, there were no large shifts between the phases. Electrolyte distribution which is important in almost every aspect of cellular function remained virtually unaffected by temperature.

This study emphasizes the point that the ionoregulatory system of rainbow trout is highly sensitive to temperature alteration, and that this sensitivity varies on a seasonal basis. It is equally apparent that these animals possess considerable ability to compensate for temperature change. The cardiovascular-respiratory systemic changes accompanying exposure to warmer temperatures should lead to net electrolyte losses. The fact that this does not occur, especially in the plasma which is in closest contact with medium, is indicative of tight regulatory control. However, the modifications observed in tissue water-electrolyte balance are not necessarily indicative of lack of tight regulatory control; what would be the use of controlling only the blood-plasma system and not the tissues. The fact that warm acclimation was generally associated with increased, rather than decreased, electrolyte levels supports the idea of electrolyte recruitment, either from the medium or diet. Since this would involve some amount of metabolic cost--the price becoming costlier as temperature rises--some degree of regulatory system destabilization might occur (Houston, 1973). The loss of regulatory precision seen at temperature extremes, i.e., fluctuations in electrolyte levels, might reflect this destabilization. However, given the complexity of the body fluid system and regulatory mechanisms, the observed variations most likely resulted from the involvement of components of differing thermosensitivity. Variations in 'passive' parameters, such as ion and/or water permeabilities, and diffusional fluxes must be considered. It is also possible that water and electrolyte parameters were 'selectively' changed by the organism to provide the 'optimum' environment for cellular function at any given temperature.

The involvement of endocrine control must also be taken into consideration especially when trying to account for the seasonal variations. Since fish kept at the same temperatures and same photoperiods exhibited changes when sampled at differing times of the year, there is obviously some sort of 'higher' coordination. It is well documented, for instance, that prolactin (secreted by the pituitary gland) is important in maintenance of sodium levels in freshwater fish (Johnson, 1973). Plasma and pituitary prolactin levels, for example, are known to vary in relation to both temperature and photoperiod (McKeown and Peter, 1976). One of the primary functions of this hormone is thought to be its role in restricting passive permeability (Ball, 1969). Cortisol has also been implicated in renal compensation for water loading at elevated temperatures, as it leads to increased urine flow in freshwater fishes (Butler, 1973). In addition, cortisol has been shown to increase net sodium uptake at the branchial level (Chan et al., 1969).

In order to account for the seasonal differences observed, it is tempting to speculate that the 'fall-winter' animals are responding to a new 'set-point' in the body fluid system. There might be seasonal cycles in circulatory hormone levels that could act to over-ride the temperature variations. However, at present, not enough work has been done in this area to allow for further discussion on the nature of hormonal involvement.

V. Conclusions

1. Estimates of extracellular phase volume and cellular electrolyte concentrations based on [^{14}C]-PEG-4000, chloride, chloride/potassium and sodium spaces were compared at three epaxial muscle sites and in cardiac muscle, liver, gut, spleen and brain samples of rainbow trout. Chloride/potassium space was determined to be a good approximation of extracellular phase volume in epaxial muscle. For cardiac muscle, gut and brain, the chloride space appeared to provide better estimates of extracellular phase volume. The sodium space was taken as providing realistic estimates of extracellular phase volume in spleen and liver.

2. The effect of thermal acclimation on the water/electrolyte balance of rainbow trout has been investigated, and a comprehensive assessment of tissue-by-tissue alterations in this balance has been provided. Water levels, and the major ions, sodium, potassium and chloride, were evaluated in the plasma, skeletal muscle, cardiac muscle, liver, spleen, gut and brain of rainbow trout acclimated to 2°, 10° and 18°C. In addition, any seasonal modifications were also examined, by sampling of 'summer' and 'late fall-early winter' groups of animals.

3. Plasma water content and electrolyte levels generally remained relatively stable across the temperature range employed. The only exceptions involved plasma sodium of 'fall-winter' animals, which increased between 2° and 10°C, and plasma potassium of the same group of animals, which rose substantially at 18°C, compared to levels at 2° and 10°C.

4. In skeletal muscle of 'summer' trout, the water content did not vary with temperature while that of 'fall' animals tended to decrease with temperature. Cold acclimation was generally associated with minimum values

for extracellular phase volume and maximum values for cellular phase volume. Tissue electrolyte levels tended to increase with temperature, most noticeably in 'fall-winter' trout. Cellular electrolyte concentrations followed the patterns observed for tissue levels of these electrolytes. Nernst equilibrium potentials did not vary with temperature in 'summer' animals, while E_{Cl} and E_K of 'fall' fish became less negative at 18°C. Seasonal differences in many of these parameters were evident. Regional differences in electrolyte content and water distribution were observed along the epaxial muscle band.

5. Cardiac muscle water content remained relatively stable across the temperature range used in both 'fall' and 'summer' groups of trout. Extracellular phase volume tended to increase with temperature, while cellular phase volume of 'fall' fish decreased at high temperature and that of 'summer' animals did not vary significantly. Electrolyte levels of 'summer' animals, both tissue and cellular, did not vary significantly with temperature. 'Fall' cardiac muscle sodium and chloride levels tended to increase with temperature while potassium exhibited maximum concentration at 10°C. Equilibrium potentials remained essentially constant with temperature, the only exception being E_K at 18°C in 'fall' series fish. Seasonal differences were apparent, with 'summer' animals having generally higher levels of electrolytes than the 'fall-winter' group.

6. Liver was notable for having the lowest water content of all tissues tested. Extracellular phase volume of 'summer' animals was of maximum value at 10°C, while cellular phase volume was at a minimum at the same temperature. In 'fall' animals, extracellular phase volume declined with cold acclimation, while cellular phase volume increased. Tissue sodium and chloride of 'summer' trout displayed maximum concentrations at 10°C;

potassium increased at 10° over levels at 2°C. 'Fall' levels of tissue sodium and chloride increased at 18°C, to maximum levels while potassium did not vary significantly with temperature. Cellular chloride and potassium generally increased with temperature. Negative results were obtained for cellular sodium. E_{Cl} of 'summer' and 'fall' trout liver increased in negativity at 2°C. 'Summer' E_K decreased in negativity at 2°C, while that of 'fall' fish decreased at 18°C. Seasonal differences were observed in tissue water content and electrolyte levels.

7. Spleen was the only tissue in which water content increased with temperature. Extracellular phase volume was highest at 18°C, while cellular phase volume was increased at 10°C compared to values at 2°C. Tissue levels of sodium and chloride increased with temperature, while potassium remained relatively stable. Cellular chloride concentrations also increased with temperature, while potassium did not vary. Estimates of cellular sodium yielded negative results. E_{Cl} increased in negativity at 2°C, while E_K decreased at 18°C.

8. Water content of gut did not vary with temperature, nor did the extracellular phase volume of 'summer' trout. 'Fall' extracellular phase volume decreased with cold acclimation while cellular phase volume of both groups increased under the same conditions. Tissue and cellular electrolytes of 'summer' trout showed no significant variation with temperature. 'Fall' tissue sodium levels also showed no variation, but tissue chloride increased between 2° and 18°C, and potassium decreased between 10° and 18°C. Cellular chloride levels of 'fall' animals reached minimum levels with cold acclimation, while potassium concentrations were at maximum values at 10°C. Like spleen and liver, estimates of cellular sodium lead to negative results. In 'summer' animals, equilibrium potentials did not vary with

temperature; 'fall' E_{Cl} reached maximum negativity at 2°C and E_K reached minimum negativity at 18°C. Seasonal variations also existed with 'fall' ECPV and electrolyte levels (with exception of potassium) being higher than those of 'summer' trout.

9. Brain water content of 'fall' fish reached maximum levels at 10°C, while that of 'summer' fish decreased at 10°C, compared to levels at 18°C. ECPV of both seasonal groups generally increased with temperature; the converse was true of the cellular phase volume. Brain sodium content of 'summer' trout did not vary with temperature, while chloride and potassium levels increased with warm acclimation. Tissue sodium and chloride of 'fall' animals declined with cold acclimation, and potassium was elevated at 10°C compared to values at 2°C. Cellular electrolyte concentrations in both series of fish generally followed the pattern observed for the tissue levels. E_{Na} was essentially constant across temperature range employed in both groups, as was 'summer' E_{Cl} and E_K. E_{Cl} of 'fall' trout brain became less negative at 10°C, while the same was true of E_K at 18°C. Only potassium concentrations differed on a seasonal basis, with 'summer' levels being much higher than those of 'fall' animals.

10. Despite the alterations observed in water content and distribution, and cellular and tissue electrolyte concentrations, temperature appeared to have little or no effect on the distribution of electrolytes between the cellular and extracellular phase, a fact that is of some importance with regard to general cellular function.

11. Seasonal differences in the response to thermal acclimation were very apparent. 'Summer' series rainbow trout appeared to have better ability to maintain their water electrolyte balance. 'Fall' animals displayed the most variation with temperature.

Bibliography

Ahokas, R.A. and F.G. Duerr (1975) Tissue water and intracellular osmoregulation in two species of euryhaline teleosts, Culea inconstans and Fundulus diaphanus. Comp. Biochem. Physiol., 52A: 449-454.

Atallah, K., P. Rauschenbach, H. Simon, F. Berthold and W. Kolbe (1977) Determination of liquid scintillation counting efficiency of ^{3}H- and/or ^{14}C-labelled samples independently of degree of colour and/or chemical quenching. Z. Naturforsch., 32: 143-149.

Behrisch, H. W. (1972) Molecular mechanisms of adaptation to low temperature in marine poikilotherms. Marine Biology, 13: 267-275.

Beyenbach, K. W. and L. Kirschner (1976) The unreliability of mammalian glomerular markers in teleostean renal studies. J. Exp. Biol., 64: 369-378.

Bolaffi, J.L. and H.E. Booke (1974) Temperature effects on LDH isozyme distribution in skeletal muscle of Fundulus heteroclitus. Comp. Biochem. Physiol., 48B: 557-564.

Bundy, H.F. (1977) Carbonic anhydrase. Comp. Biochem. Physiol., 57B: 1-7.

Bygrave, L. L. (1967) Ionic environment and metabolic control. Nature, 214: 667-671.

Byrne, J., F. W. Beamish and R. L. Saunders (1972) Influence of salinity, temperature and exercise on plasma osmolality and ionic concentration in Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can., 29: 1217-1220.

Caldwell, R.S. (1969) Thermal compensation of respiratory enzymes in tissues of goldfish. Comp. Biochem. Physiol., 31: 79-93.

Cameron, J.N. (1976) Branchial ion uptake in Arctic grayling: Resting values and effects of acid-base disturbances. J. Exp. Biol., 64: 711-725.

Cameron, J.N. and J.C. Davis (1970) Gas exchange in rainbow trout with varying blood oxygen capacity. J. Fish. Res. Bd. Can., 27: 1069-1085.

Catlett, R.H. and D.R. Millich (1976) Intracellular and extracellular osmoregulation of temperature acclimated goldfish: Carassius auratus. Comp. Biochem. Physiol., 55A: 261-269.

Chan, D.K.O. and T.M. Wong (1977) Physiological adjustments to dilution of the external medium in the lip shark, Hemiscyllium plagiosiem (Bennett) 1. Size of body compartments and osmolyte compositions. J. Exp. Zool. 200: 71-84.

Conte, F.P. (1969) Salt secretion. In Fish Physiology. (Hoar, W. and D. Randall, Editors). Vol. 1. Academic Press, New York. pp. 241-292.

Conway, E.J. (1957) The nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol. Rev., 37: 84-132.

Cotlove, E., and C.A. Hogben (1962) Chloride. In Mineral Metabolism (Comar, E. and F. Bronner, Editors). Vol. IIB. Academic Press, New York. pp. 109-173.

Crawshaw, L. I. (1977) Physiological and behavioural reactions of fishes to temperature changes. J. Fish. Res. Bd. Can., 34: 730-734.

Daniel, E. E. (1958) Smooth muscle electrolytes. Can. J. Biochem. Physiol., 36: 805-818.

Das, A.B. (1967) Biochemical changes in tissues of goldfish acclimated to low and high temperatures. II. Synthesis of protein and RNA of subcellular fractions and tissue composition. Comp. Biochem. Physiol., 21: 469-485.

Das, A.B. and C.L. Prosser (1967) Biochemical changes in tissues of goldfish acclimated to high and low temperature. I. Protein synthesis. Comp. Biochem. Physiol., 21: 449-467.

Davis, J.C. (1968) Influence of temperature and activity on certain cardiovascular and respiratory parameters in adult sockeye salmon. M.Sc. Thesis, University of British Columbia (cited in Randall, 1970).

Dean, J.M. (1969 Metabolism of thermally-acclimated trout. Comp. Biochem. Physiol., 29: 185-196.

De Renzis, G. and J. Maetz (1973) Studies on the mechanism of chloride absorption by the goldfish gill. Relation with acid-base regulation. J. Exp. Biol., 59: 339-358.

Evans, D. H. (1969) Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. J. Exp. Biol., 50: 689-703.

Forbes, G.B. (1962) Sodium. In Mineral Metabolism (Comar, B. and F. Bronner, Editors) Vol. IIB. Academic Press, New York. pp. 2-72.

Fry, F.E.J. (1971) Effect of environmental factors on the physiology of fish. In Fish Physiology. (Hoar, W. and D. Randall, Editors) Vol. VI. Academic Press, New York. pp. 1-98.

Fry, F. E. J. and P. Hochachka (1970) Fish. In Comparative Physiology of Thermoregulation. (Whittow, G. C., Editor) Vol. I. Academic Press, New York. pp. 79-134

Giles, M.A. and W.E. Vanstone (1976) Changes in ouabain sensitive adenosine triphosphatase acticity in gills of coho salmon (Oncorhynchus kisutch) during parr-smolt transformation. J. Fish. Res. Bd. Can., 33: 56-42.

Gordon, M. S. (1959) Ionic regulation in the brown trout, Salmo trutta L. J. Exp. Biol., 36: 227-252.

Grigg, G. C. (1969) Temperature induced changes in oxygen equilibrium curve of the blood of brown bullhead Ictalurus nebulosus. Comp. Biochem. Physiol., 28: 1203-1223.

Hazel, J. (1972) Effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial muscle of the common goldfish (Carassius auratus L.) I. Properties of the enzyme and the effect of lipid extraction. Comp. Biochem. Physiol., 43B: 837-861.

Hazel, J. and C. L. Prosser (1974) Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev., 54: 620-667.

Heinicke, E. and A. H. Houston (1965a) A note on water balance in goldfish during lethal heat shock. Can. J. Zool., 43: 847-852.

Heinicke, E. and A. H. Houston (1965b) Effect of thermal acclimation and sublethal heat shock on ionic regulation in goldfish. J. Fish. Res. Bd. Can., 22: 1455-1476.

Herberg, J. (1960) Determination of carbon-14 and tritium in blood and other whole tissues: Liquid scintillation counting of tissues. Analytical Chemistry, 32: 42-46.

Hickman, C. P. (1964) Seasonal change in muscle electrolyte balance in the white sucker, Catastomus commersoni. Am. Zool., 4: 284.

Hickman, C. P. (1965) Studies on renal function in freshwater teleost fish. Trans. Roy. Soc. Canada, Sect. III (4), 3: 213-236.

Hickman, C.P., R.A. McNable, J.S. Nelson, E.D. Van Breeman and D. Comfort. (1964) Effect of cold acclimation on electrolyte distribution in rainbow trout, Salmo gairdneri. Can. J. Zool., 42: 577-597.

Hickman, C.P. and B.F. Trump (1969) The kidney. In Fish Physiology (Hoar, W. and D. Randall, Editors). Vol. I. Academic Press, New York. pp. 91-239.

Hochachka, P.W. and F.R. Hayes (1962) The effect of temperature acclimation on pathways of glucose metabolism in trout. Can. J. Zool., 40: 261-270.

Hochachka, P.W. and G. Somero (1971) Biochemical adaptation to the environment. In Fish Physiology (Hoar, W. and D. Randall, Editors). Vol. VI. Academic Press, New York. pp. 100-156.

Hochachka, P.W. and G. Somero (1973) Strategies of Biochemical Adaptation. W.B. Saunders, Toronto.

Houston, A. H. (1973) Environmental temperature and the body fluid system of teleosts. In Responses of Fish to Environmental Changes (Chavin, E., Editor). Charles C. Thomas, Springfield. pp. 87-162.

Houston, A. H. and J. C. Fenwick (1964) Thermoacclimatory variations in the plasma proteins of the goldfish, Carassius auratus. Am. Zool., 5: 695.

Houston, A. H. and J. Madden (1968) Environmental temperature and plasma electrolyte regulation in the carp, Cyprinus carpio. Nature, 217: 969-970.

Houston, A. H., J. A. Madden and M. A. DeWilde (1970) Environmental temperature and body fluid system of freshwater teleost. IV. Water-electrolyte regulation in thermally acclimated carp, Cyprinus carpio. Comp. Biochem. Physiol., 34: 805-818.

Houston, A.H., K.M. Mearow and J.S. Smeda (1976) Further observations upon the hemoglobin systems of thermally-acclimated freshwater teleosts. Comp. Biochem. Physiol., 54A: 267-273.

Houston, A.H., R. Reaves, D. A. Madden and M. A. DeWilde (1968) Environmental temperature and body fluid system of the freshwater teleost I. Ionic regulation in thermally acclimated rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 25: 563-581.

Houston, A. H., J. A. Madden, R. J. Woods and H. Miles. (1971) Variations in blood and tissue chemistry of brook trout Salvelinus fontinalis, subsequent to handling, anesthesia and surgery. J. Fish. Res. Bd. Can., 28: 635-642.

Isaia, J. (1972) Comparative effects of temperature on the sodium and water permeabilities of the gills of a stenohaline freshwater fish (Carassius auratus), and stenohaline marine fish (Serranus scriba, Serranus cabrilla). J. Exp. Biol. 56: 263-272.

Jampol, L. and F. Epstein (1970) Sodium-potassium activated adenosine triphosphatase and osmotic regulation by fishes. Am. J. Physiol., 218: 607-611.

Johnson, D. W. (1973) Endocrine control of hydromineral balance in teleosts. Am. Zool., 13: 799-818.

Johnson, S. L., R. D. Ewing and J. A. Lichatowich (1977) Characterization of gill (Na^+-K^+)-activated adenosine triphosphatase from Chinook salmon, Oncorynchus tshawytscha. J. Exp. Zool., 199: 345-354.

Kanungo, M. S. and C. L. Prosser (1959) Physiological and biochemical adaptation of goldfish to cold and warm temperatures. II. Oxygen consumption of liver homogenate; oxygen consumption and oxidative phosphorylation of liver mitochondria. J. Cell. Comp. Physiol. 54: 265-274.

Katzman, R. S. (1976) Maintenance of a constant brain extracellular potassium. Fed. Proc., 35: 1243-1247.

Kerstetter, T. and L. Kirschner (1972) Active chloride transport by the gills of rainbow trout, Salmo gairdneri. J. Exp. Biol., 56: 263-272.

Knipprath, W. E., and J. F. Mead (1968) The effect of environmental temperature on the fatty acid composition and on the in vivo incorporation of 1-^{14}C-acetate in goldfish Carassius auratus. Lipids, 2: 121-128.

Knoebel, L. K. (1971) Secretion and action of digestive juices; Absorption. In Physiology (Selkurt, E., Editor). Little, Brown and Company, Boston. pp. 599-650.

Little, J. R. (1964) Determination of water and electrolytes in tissue slices. Anal. Biochem., 7: 87-95.

Lutz, P. (1972) Extracellular spaces and composition of various tissues of perch. Comp. Biochem. Physiol., 41A: 77-88.

Mackay, W. C. (1974) Effect of temperature on osmotic and ionic regulation in goldfish, Carassius auratus L. J. Comp. Physiol., 88: 1-19.

Mackay, W. C. and D. D. Beatty (1968) The effect of temperature on renal function in white sucker, Catastomus commersoni. Comp. Biochem. Physiol., 26: 235-245.

Maetz, J. (1971) Fish gills: Mechanisms of salt transfer in fresh water and sea water. Phil. Trans. Roy. Soc. London B., 262: 209-249.

Maetz, J. (1972) Branchial sodium exchange and ammonia exchange in the goldfish, Carassius auratus. Effects of ammonia-loading and temperature changes. J. Exp. Biol., 56: 601-620.

Maetz, J. (1974) Aspects of adaptation to hypo-osmotic and hyper-osmotic environments. In Biochemical and Biophysical Perspectives in Marine Biology. Vol. I. (Malins, W.C. and J.R. Sargent, Editors). Academic Press, New York. pp. 1-167.

Manery, J.F. (1954) Water and electrolyte metabolism. Physiol. Rev., 34: 334-417.

Maren, J. (1967) Carbonic anhydrase in the animal kingdom. Fed. Proc., 26: 1097-1103.

McCartney, J. (1976) Sodium-potassium dependent adenosine triphosphatase activity in gills and kidney of Atlantic salmon, Salmo salar. Comp. Biochem. Physiol., 53A: 351-353.

McCarty, L. S. (1977) Thermoacclimatory variations in the activities of enzymes implicated in ion transport in the rainbow trout, Salmo gairdneri. M. Sc. Thesis, Department of Biology, Brock University, St. Catharines, Ontario.

McCarty, L. S. and A. H. Houston (1977) (Na^+/K^+)- and (HCO_3^-)-stimulated ATPase activities in the gills and kidneys of thermally-acclimated rainbow trout, Salmo gairdneri. Can. J. Zool. 55: 704-712.

McIver, D. J. and A. D. Macknight (1974) Extracellular space in some isolated tissues. J. Physiol., 239: 31-49.

Motais, R. and J. Isaia (1972) Temperature dependence of permeability to water and to sodium of gill epithelium of the eel, Anguilla anguilla. J. Exp. Biol., 56: 587-600.

Newell, R. C. (1969) Effects of fluctuation in temperature on metabolism of intertidal invertebrates. Am. Zool. 9: 293-307.

Murphy, P. G., and A. H. Houston. (1974) Environmental temperature and the body fluid system of the freshwater teleost. V. Plasma electrolyte levels and branchial microsomal (Na^+-K^+)-ATPase in thermally-acclimated goldfish, Carassius auratus. Comp. Biochem. Physiol., 47B: 563-570.

Murphy, P. G., and A. H. Houston (1977) Temperature, photoperiod and water-electrolyte balance in rainbow trout, Salmo gairdneri. Can. J. Zool., 55: 1377-1388.

Parvatheswararao, V. (1967) Some mechanisms underlying thermal acclimation in a freshwater fish, Etroplus maculatus (Teleostei). Comp. Biochem. Physiol., 21: 619-626.

Paschen, K., and C. Fuchs (1971) A new micro-method for Na^+, K^+, Ca^{2+} and Mg^{2+} determinations in a single serum dilution by atomic absorption spectrophotometry. Clin. Chem. Acta, 35: 401-408.

Pedley, J.A., R. Fisher, K. Futamachi and D. Price (1976) Regulation of extracellular potassium concentration in epileptogenesis. Fed. Proc., 35: 1254-1259.

Pora, E. A. and O. Prekup (1960) The study of excretory processes of freshwater fish. II. The effect of environmental temperature on the excretory processes of carp and Crucian carp. Vopr. Ikhatiol., 15: 138-147 (Biol. Abstr., 37, No. 13391, 1962; cited in Houston, 1973).

Powers, D. A. (1974) Structure, function and molecular ecology of fish hemoglobins. Ann. N. Y. Acad. Sci., 241: 472-490.

Prosser, C. L. (1962) Acclimation of poikilothermic vertebrates to low temperature. In Comparative Physiology of Temperature Regulation (Hannon, J. B., and E. Vierecki, Editors). Arctic Aeromedical Laboratory, Ft. Wainwright, Alaska. pp. 1-34.

Prosser, C. L. (1973) Comparative Animal Physiology. 3rd edition. W. B. Saunders Co. 966 p.

Prosser, C. L., W. Mackay and K. Kato (1970) Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. Physiol. Zool., 43: 81-89.

Ramirez-Munoz, J. (1968) Atomic Absorption Spectrophotometry. Elsevier Publishing Company. p. 269.

Randall, D. J. (1968) The functional morphology of the heart in fishes. Am. Zool. 8: 179-189.

Randall, D. J. (1970) Gas exchange in fish. In Fish Physiology (Hoar, W., and D. J. Randall, Editors). Vol. IV. Academic Press, New York. pp. 253-292.

Randall, D. J., G. F. Holeton and E. Don Stevens (1967) The exchange of oxygen and carbon dixoide across the gills of rainbow trout. J. Exp. Biol., 46: 339-348.

Randall, D. J., D. Baumgardner and M. Malyusz (1972) The relationship between gas and ion transfer across the gills of fishes. Comp. Biochem. Physiol., 41A: 629-637.

Reaves, R. S., A. H. Houston and J. A. Madden (1968) Environmental temperature and the body fluid system of freshwater teleosts. II. Ionic regulation in rainbow trout following abrupt thermal shock. Comp. Biochem. Physiol. 25: 848-860.

Roots, B. I. (1968) Phospholipids of goldfish (Carassius auratus) brain and the influence of environmental temperature. Comp. Biochem. Physiol. 25: 457-466.

Schmidt-Nielsen, B. and J. L. Renfro (1975) Kidney function of the American eel, Anguilla rostrata. Am. J. Zool., 228: 420-431.

Schmidt-Nielsen, B., J.L. Renfro and D. Benos (1972) Estimation of extracellular space and intracellular ion concentrations in osmo-conformers, hypo- and hyperosmoregulators. Bull. Mt. Desert Island Biol. Lab., 12: 99-104.

Shaklee, J. B., J. A. Christiansen, B. O. Sidell, C. L. Prosser and G. S. Whitt (1977) Molecular aspects of temperature acclimation in fish: contributions of changes in enzyme activities and isozyme patterns to metabolic reorganization in the green sunfish. J. Exp. Zool., 201: 1-20.

Sidell, B. D., F. R. Wilson, J. Hazel and C. L. Prosser (1973) Time course of thermal acclimation in goldfish. J. Comp. Physiol., 84: 119-127.

Smeda, J. S. and A. H. Houston (1978) Carbonic anhydrase (azetazolamide-sensitive esterase) activity in the red blood cells of thermally-acclimated rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., in press.

Smith, L. S. and G. R. Bell (1967) Anesthetic and surgical technique for Pacific salmon. J. Fish. Res. Bd. Canada, 24: 1579-1588.

Soivio, A. and A. Oikari (1976) Hematological effects of stress on a teleost, Esox lucius L. J. Fish. Biol., 8: 397-411.

Somero, G. (1969) Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. Am. Nat., 103: 517-530.

Somero, G. and P. W. Hochachka (1968) Effect of temperature on catalytic and regulatory functions of pyruvate kinases of rainbow trout and Antarctic fish, Trematomus bernacchi. Biochem. J., 110: 395-400.

Sutton, A. H. (1968) The relationship between ion and water contents of cod (Gadus morhua) muscle. Comp. Biochem. Physiol., 24: 149-161.

Toews, D. P. and C. P. Hickman (1969) The effect of cycling temperatures on electrolyte balance in skeletal muscle and plasma of rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 29: 905-918.

Towle, D. W., M. E. Gilman, and J. D. Hempel (1977) Rapid modulation of gill Na^+-K^+-dependent ATPase activity during acclimation of killifish to salinity change. J. Exp. Zool., 202: 179-186.

Umminger, B. L. (1970) Osmoregulation by the killifish Fundulus heteroclitus, in freshwater at temperatures near freezing. Nature, Lond., 225: 294-295.

Umminger, B. L. (1971) Patterns of osmoregulation in freshwater fishes at temperatures near freezing. Physiol. Zool. 44: 20-27.

Ward, W. E., and R. M. Stokes (1969) Estimation of extracellular space in intestinal sections in vitro from rainbow trout. Comp. Biochem. Physiol., 28: 981-983.

Watson, T. (1978) Zinc effects on branchial adenosine triphosphatases and blood constituents of rainbow trout, Salmo gairdneri. Ph.D. Thesis, Department of Zoology, University of Guelph, Guelph, Ontario.

Williams, J. A. and D. M. Woodbury (1971) Determination of extracellular space and intracellular electrolytes in rat liver in vivo. J. Physiol., 212: 85-99.

Wilson, F. R., G. Somero and C. L. Prosser (1974) Temperature-metabolism relations of two species of Sebastes from different thermal environments. Comp. Biochem. Physiol., 47B: 485-491.

Wilson, F. R., M. S. Champion, G. S. Whitt and C. L. Prosser (1975) Isozyme patterns in tissues of temperature acclimated fish. In Isozymes II, Physiologic Function. Academic Press, New York. pp. 193-206.

Yager, P. (1977) A novel mechanism for the (Na^+-K^+)-ATPase. J. Theor. Biol., 66: 1-11.

Appendix I

Natural History of the Rainbow Trout

(a) Distribution

The rainbow trout, Salmo gairdneri, is widely distributed in North America. The native range of the rainbow trout includes the eastern Pacific Ocean and the fresh-water mainly west of the Rocky Mountains, from Northwest Mexico to Alaska. Because it has been widely introduced outside its natural range, this species now occurs in practically all suitable locations. In Canada, the rainbow trout can be found across the country, in British Columbia, Alberta, central Manitoba, across southern portions of Ontario, Quebec, New Brunswick and Nova Scotia, and in Newfoundland (Scott and Crossman, 1973).

(b) Biology

Rainbow trout are basically spring spawners and usually reproduce in small river tributaries or streams from March to August, but mainly from mid-April to late June. Great Lakes populations may enter spawning streams in late October to early May, and usually spawn from late December to late April. Water temperature at spawning is usually between 10° and 15°C.

The upper lethal temperature for rainbow trout is between 24° and 25°C, depending on the temperature of acclimation (Brett, 1956; Scott and Crossman, 1973). The lower lethal temperature for trout is 0-0.5°C, again depending on the acclimation temperature. 10°C is generally considered to be an optimum temperature, since growth and food utilization seem to be greatest at this temperature. Final preferred temperature has been calculated as 13°C by Garside and Tait (1958), while Scott and

Crossman (1973) suggest rainbow trout are most successful in habitats with temperatures of 21°C or lower, as long as there is cooler, well-oxygenated water into which they can retreat.

It is possible that lake populations of trout may encounter the temperatures tested here sometime throughout their lifetime--since many lakes are known to warm up to 20°C or over during summer months and fall to near 0°C in winter months (Scott and Crossman, 1973).

Appendix II

Operation Procedure:

(a) Anesthetic:

100 mg/1 MS222-Tricaine methane sulfonate (Sigma Chemicals). This must be freshly prepared for each use.

(b) Determination of depth of anesthesia:

Deep anesthesia-- no response to pinching of anal fin.

Shallow anesthesia--slight quivering response to same stimulation

Very shallow anesthesia--whole fish responds

The fish is fully anesthetized when opercular movements stop (Smith and Bell, 1967).

The fish were removed from the anesthetic after the first flaring of the operculum, an indication that opercular movements are about to cease. The total time in the anesthetic bath was approximately 3 to 4 minutes.

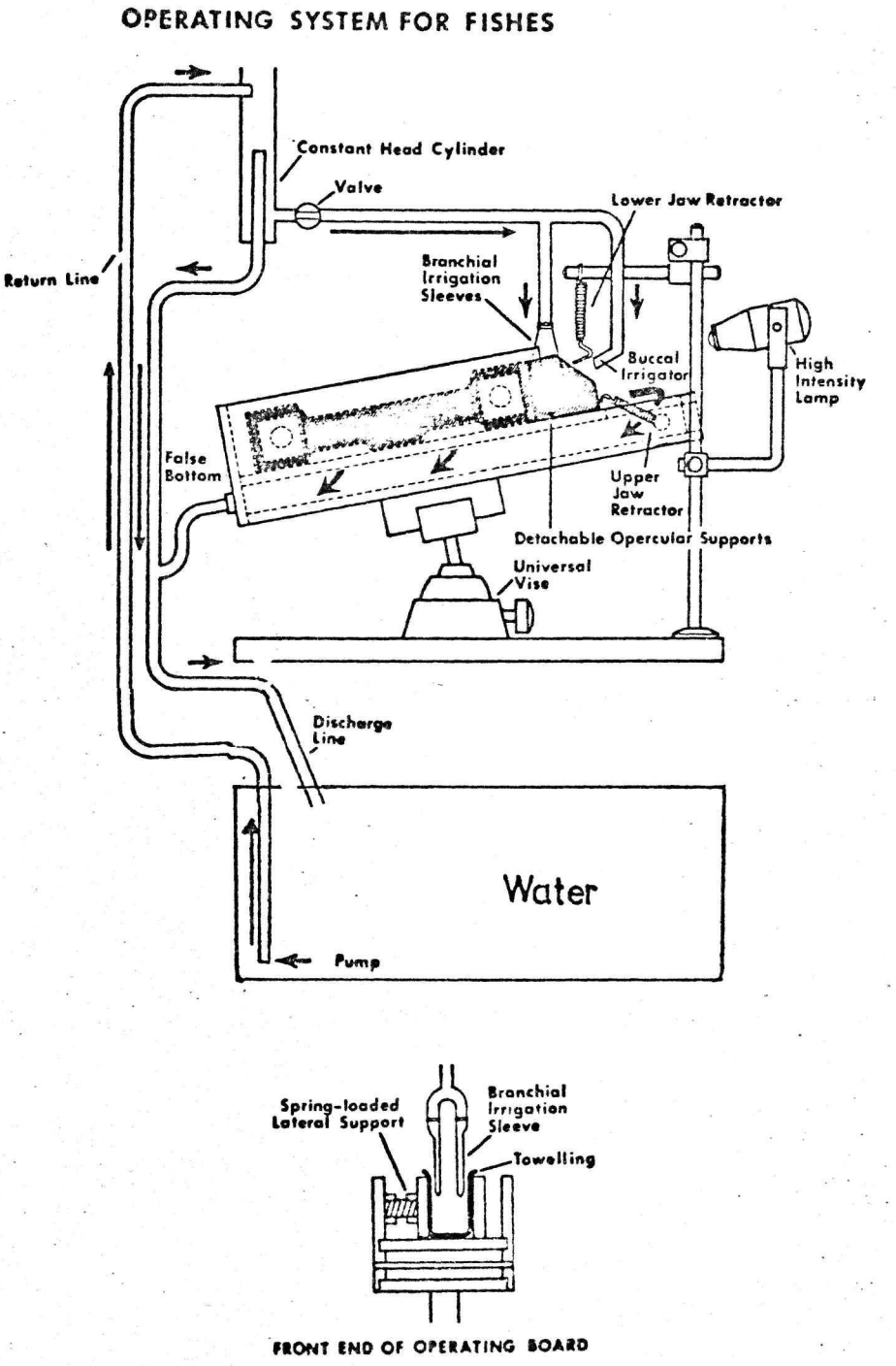
(c) Catheterization:

After removal from the anesthetic, fish were placed ventral side up in an operating apparatus, which consists of a restraining cradle and a water reservoir which provides for constant branchial irrigation (see Appendix Figure 1). It was necessary to add a small amount of anesthetic to this reservoir.

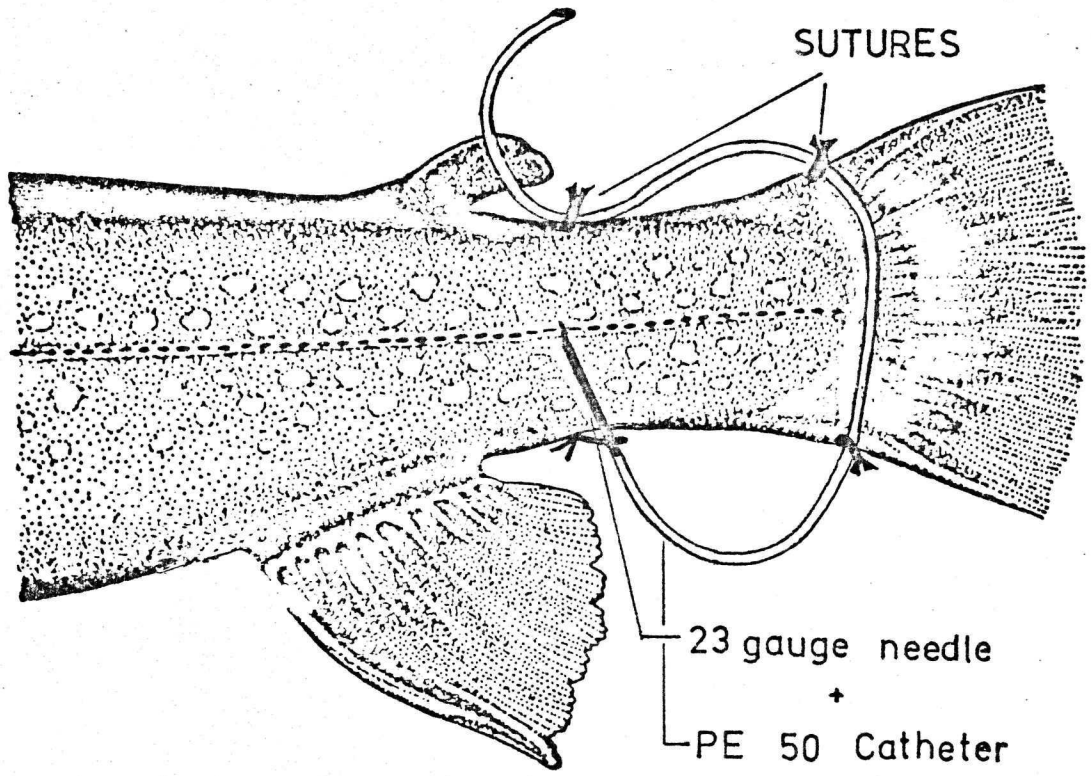
Catheters consisted of a length of polyethylene tubing (PE-50), approximately 45 cm long, equipped with a short length (approximately 2 cm) of 23 gauge needle with a deflected point (see Appendix Figure 2)

The catheter was inserted into the caudal artery from the ventral surface. Prior to insertion, the catheter was filled with sterile saline

Appendix Fig. 1



Appendix Fig. 2



(0.7%). When it is correctly placed, the blood pressure forces the saline out of the catheter. The catheter was then flushed with a heparin/saline solution (1:4, v:v) to ensure that the needle was not blocked and, refilled with this solution and then sealed using a vinyl plastic putty (Critoseal, normally used to seal hematocrit tubes).

The tubing was then anchored to the body and caudal fin in three places, using silk sutures (Figure 2). Sutures were set in place prior to catheterization. The catheterization process was usually completed within 3 to 4 minutes, and the entire operation, from netting of fish to replacement in holding tanks, was normally within 6 to 8 minutes.

The advantage of this technique, as compared to others used, is that it is simple and quick, and can be completed by one person.

Appendix III:

Liquid Scintillation Solutions:

(i) Tissue Solubilizer--NCS (Amersham/Searle)

NCS is a solution of quaternary ammonium base in toluene. 1.5 ml of this was added to each vial.

(ii) Decolorizer

The decolorizer was a freshly prepared solution of benzoyl peroxide in toluene. In order to dissolve 1.0 g benzoyl peroxide in 5.0 ml of toluene, it is necessary to heat the solution to 60°C. Once dissolved, the solution was cooled and filtered. 0.2 ml of this was added to each vial.

(iii) Scintillant

ACS (aqueous counting scintillant) (Amersham/Searle) was mixed with p-xylene (scintillation grade) in a 2:1 ACS:xylene ratio. 15.0 ml of the mixture were added to each vial.

(iv) [^{14}C]-polyethylene glycol (mol. wt. 4000) (Amersham/Searle Corp.)

[^{14}C]-PEG-4000 used had a specific activity of 54 μCi/gram. The compound was in the form of a freeze-dried solid (1.0 g) and was prepared for use by dissolving it in 1.0 ml of 0.7% saline. The solution so obtained has an activity of 250 μCi/ml. The injected dose was 25 μl of this solution.

By using the ESR given for each sample, the counting efficiency of the sample was estimated and the cpm corrected accordingly.

(vi) Vial Contents:

Tissue Samples:

Tissue sample	≤0.2 g
NCS solubilizer	1.5 ml
Decolorizer	0.2 ml
Scintillant	15.0 ml

Plasma Samples:

Plasma	100 µl
NCS solubilizer	1.5 ml
Decolorizer	0.2 ml
Scintillant	15.0 ml

Blanks:

Saline	100 µl
NCS solubilizer	1.5 ml
Decolorizer	0.2 ml
Scintillant	15.0 ml

Appendix IV

Appendix Table 1a. Raw data for rainbow trout acclimated to 10°C (season evaluation study) - physical characteristics and plasma electrolyte and water content.

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/kg)
1	M	31.0	380.5	130.93	3.58	108.12	925.31
2	M	30.0	338.5	110.92	4.64	142.80	885.16
3	F	30.5	335.5	134.36	3.23	130.43	975.33
4	F	36.0	427.0	131.82	3.25	139.85	970.80
5	M	30.0	350.0	137.36	3.60	139.95	971.16
6	M	29.0	311.0	134.59	4.48	135.44	958.80
7	M	30.0	352.5	137.36	3.30	138.43	962.13
8	M	28.5	303.5	132.36	4.04	129.43	957.93
9	M	30.0	326.5	136.25	3.93	127.17	943.49
10	F	29.0	238.0	131.21	4.34	138.05	966.33
11	F	30.0	261.0	129.05	4.05	123.41	974.44
12	M	30.0	253.5	110.77	2.98	125.67	977.27
13	M	31.0	304.0	130.16	3.75	141.45	964.74
14	F	30.0	301.0	135.09	3.75	147.47	949.40
15	F	31.0	331.0	133.98	2.93	81.39	954.29
16	M	30.0	297.0	131.82	3.51	150.93	950.15
17	M	28.0	255.0	121.85	3.56	141.45	966.19
18	F	28.5	240.0	103.57	3.53	96.36	978.61
19	F	29.5	252.5	108.00	2.68	156.19	933.79
20	M	30.0	297.0	81.96	2.28	114.39	987.74
21	F	28.0	231.0	135.70	3.11	130.18	972.75
22	M	28.5	245.0	143.46	3.75	150.47	974.05
23	M	27.0	223.0	126.28	3.11	162.40	971.83
24	F	28.0	236.0	101.35	2.93	108.36	982.14
25	M	30.5	400.0	155.79	2.92	129.85	---
26	M	27.0	221.5	160.39	2.88	123.27	---
27	M	27.0	294.0	132.36	2.04	124.41	---
28	M	31.5	410.5	137.06	2.47	115.00	---
29	M	27.0	270.5	142.41	2.75	125.05	---
30	F	30.0	361.5	152.66	1.14	122.60	---
31	F	30.5	366.0	152.41	2.33	111.23	---
32	F	29.0	259.0	154.33	5.95	132.80	---
33	M	30.0	380.0	146.05	2.25	121.80	---
MEAN		29.5	309.0	134.34	3.27	128.24	966.30
M_1*		28.9	282.9	127.76	2.94	122.53	962.05
M_2**		29.9	325.1	140.92	3.60	133.94	970.50

Appendix Table 1b. Raw data for post-opercular muscle of rainbow trout acclimated to 10°C

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{H2O}$ (ml/kg)
1	8.99	145.54	3.93	756.53	34.27	25.22	5.96	15.93
2	10.36	143.89	6.01	745.68	38.04	25.62	90.72	46.95
3	12.71	161.93	7.18	759.32	46.67	41.60	90.06	66.06
4	22.89	201.11	6.84	750.14	43.99	45.43	188.58	21.53
5	9.47	151.45	6.07	766.49	39.04	20.74	66.95	38.34
6	9.89	154.19	5.72	752.61	36.81	26.77	70.45	56.11
7	10.44	149.52	5.62	753.19	36.53	29.28	73.13	29.02
8	10.83	150.56	5.78	757.80	40.19	29.12	75.53	59.29
9	14.44	153.62	7.03	751.01	49.75	46.54	86.82	60.54
10	13.15	166.29	7.18	757.91	47.19	38.92	84.04	60.01
11	12.08	163.68	6.31	777.85	46.02	37.96	91.21	54.74
12	12.27	168.87	4.75	772.04	34.02	28.54	108.25	49.47
13	13.15	142.94	6.35	767.56	40.40	30.84	97.43	46.02
14	11.29	141.66	5.64	762.39	34.42	25.91	69.11	24.47
15	10.49	144.79	5.67	773.36	62.25	60.39	65.01	61.49
16	11.89	145.55	6.07	765.36	41.72	32.12	85.70	34.33
17	9.26	148.59	5.18	768.98	32.96	23.43	72.58	33.14
18	8.35	140.12	4.93	791.29	46.05	36.15	78.89	16.11
19	11.95	152.19	6.36	777.05	43.03	37.72	106.84	38.07
20	8.05	145.26	4.99	774.00	39.26	35.66	95.05	23.25
21	19.67	137.70	4.85	757.53	33.55	25.29	141.00	28.23
22	10.59	147.01	5.61	750.06	33.55	23.03	71.90	48.77
23	7.29	154.24	5.16	762.39	28.58	20.79	59.72	20.69
24	7.17	149.95	4.75	771.06	29.46	32.91	68.99	22.99
25	14.69	146.83	7.21	762.69	50.51	46.11	82.38	54.94
26	11.34	148.31	7.06	777.79	49.24	45.68	79.03	69.33
27	12.71	148.26	7.52	784.12	54.42	50.97	110.04	56.66
28	10.69	139.90	5.75	771.37	44.26	39.25	81.79	28.28
29	12.99	141.25	6.56	769.16	47.21	45.49	90.17	50.62
30	11.65	150.93	6.22	760.21	44.25	45.40	81.01	46.12
31	12.93	142.65	5.92	754.47	39.62	42.46	82.27	45.29
32	12.24	146.89	6.76	764.55	45.28	46.31	84.19	53.55
33	12.37	138.98	7.23	755.11	50.85	45.50	101.54	52.54
MEAN	11.26	149.96	5.99	76..57	43.48	38.35	89.57	45.84
M_1*	10.67	145.41	5.62	759.93	38.25	35.42	79.61	39.19
M_2**	12.36	154.46	6.36	767.53	48.64	41.19	98.14	48.69

M_1 = 95% Confidence Interval lower limit
M_2 = 95% Confidence Interval upper limit

Appendix Table 1c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	9.94	147.62	5.36	748.09	29.33	19.80	56.13	16.62
2	10.25	155.19	5.78	767.19	53.42	41.15	89.58	39.94
3	9.98	161.91	5.54	764.33	36.01	29.23	70.72	91.83
4	11.93	143.75	5.29	745.55	30.93	28.43	87.86	16.27
5	8.26	141.48	5.02	767.72	36.78	26.59	58.39	28.64
6	9.04	152.35	5.18	750.49	35.09	23.53	64.59	42.13
7	8.92	151.81	4.85	748.68	31.39	22.45	67.48	26.43
8	9.62	138.21	5.41	765.67	37.62	27.72	67.09	40.59
9	11.85	156.14	5.84	760.60	41.33	26.39	71.13	52.97
10	11.63	163.64	7.15	764.98	46.99	38.62	74.30	53.94
11	9.76	159.93	4.97	780.38	36.25	25.85	73.79	41.31
12	11.45	150.65	4.68	784.46	35.52	26.51	101.02	50.32
13	10.38	151.35	4.84	777.86	30.79	19.98	76.19	46.29
14	9.16	139.04	4.85	764.04	29.60	17.94	58.07	59.00
15	10.52	148.92	4.74	774.95	52.46	47.38	65.83	50.34
16	10.74	142.74	5.23	767.66	36.64	27.22	77.41	28.88
17	8.19	150.11	4.86	759.74	27.10	17.85	64.94	24.71
18	8.81	150.72	4.70	774.12	39.23	30.63	83.24	21.25
19	9.75	147.71	5.59	765.66	38.94	31.05	80.83	36.36
20	7.36	145.56	4.40	770.19	34.62	29.83	86.90	24.23
21	17.54	141.54	4.46	748.42	30.82	22.68	125.73	23.61
22	19.50	152.65	4.85	735.15	29.01	19.81	158.39	37.59
23	6.15	153.70	5.62	740.95	31.13	24.19	47.85	19.86
24	5.56	138.59	4.62	784.02	38.37	30.62	53.88	17.94
25	12.81	146.83	7.21	761.92	50.21	46.29	87.89	49.13
26	10.79	144.37	7.06	769.18	49.54	45.20	71.42	57.00
27	13.83	143.61	6.91	746.34	49.99	49.53	111.04	44.16
28	10.63	124.05	4.28	772.87	33.47	26.29	82.33	31.41
29	10.65	153.46	4.68	769.18	33.68	27.86	79.24	37.62
30	8.86	169.26	5.05	765.19	37.01	38.32	60.22	32.25
31	10.26	146.28	4.98	757.67	40.29	37.05	71.46	32.76
32	10.21	144.25	5.19	764.25	38.04	18.95	70.23	27.29
33	12.27	143.59	6.11	761.21	41.72	38.76	89.19	41.11
MEAN	10.41	148.78	5.24	761.78	37.14	29.53	77.36	35.96
M_1*	9.43	145.55	4.92	757.28	34.81	26.31	70.50	31.62
M_2**	11.39	152.00	5.56	766.28	39.47	32.75	84.22	40.31

Appendix Table 1d. Raw data for caudal muscle of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	15.98	126.69	9.73	772.01	84.85	81.02	117.25	83.86
2	15.64	149.54	9.92	758.29	62.79	54.36	136.95	133.59
3	24.27	147.52	15.61	783.27	101.47	102.58	171.98	137.42
4	18.91	146.05	10.63	765.07	68.36	68.47	139.26	26.30
5	13.89	145.39	10.56	783.87	67.91	62.55	98.70	43.39
6	15.39	150.52	9.72	766.43	64.59	57.01	109.64	92.73
7	17.07	145.43	9.98	769.99	64.87	60.65	119.56	103.18
8	14.45	122.60	18.25	785.48	126.91	123.24	100.65	160.07
9	16.28	155.14	8.92	766.29	65.13	60.79	97.38	83.94
10	14.31	166.53	9.13	763.55	60.00	53.51	91.45	61.29
11	13.94	147.21	7.82	782.88	57.10	48.67	105.26	47.11
12	21.60	138.59	12.05	788.89	86.29	85.22	---	80.46
13	15.43	141.59	8.86	779.59	56.37	48.46	114.37	89.70
14	19.16	136.63	11.66	780.82	71.16	65.06	117.39	107.40
15	12.74	141.79	8.49	793.06	---	---	---	---
16	12.13	150.65	7.18	765.45	49.35	41.15	87.43	42.33
17	15.38	143.52	8.55	766.68	54.46	47.60	125.92	35.68
18	20.09	119.39	11.37	795.97	105.26	104.69	---	96.87
19	17.73	122.95	11.14	790.86	92.62	71.11	---	---
20	22.69	97.81	15.39	788.99	---	---	---	---
21	28.95	139.81	8.75	764.63	60.49	54.42	---	81.74
22	19.44	135.93	9.69	786.20	57.84	50.09	131.99	93.45
23	16.71	128.38	16.94	793.68	91.61	89.76	148.09	126.79
24	9.29	131.59	7.54	792.22	69.95	56.48	94.87	77.59
25	13.79	146.39	9.99	779.90	56.64	53.55	93.77	53.26
26	13.38	130.27	6.98	775.89	44.52	42.41	88.56	69.04
27	13.92	131.03	8.19	754.75	59.75	60.47	112.29	62.41
28	15.46	111.49	---	812.29	---	---	119.74	92.27
29	9.87	137.63	7.82	779.79	52.40	50.81	93.57	94.05
30	9.12	166.98	6.41	780.57	44.05	46.19	68.42	82.39
31	14.92	130.98	7.30	788.61	50.07	48.63	92.85	48.71
32	11.85	130.41	8.39	772.98	69.15	56.58	81.51	30.07
33	15.30	136.79	7.00	769.74	48.79	45.68	96.82	59.49
MEAN	15.76	139.63	9.50	775.86	67.41	63.09	107.76	75.15
M_1*	14.11	134.21	8.24	772.19	60.49	55.69	98.19	64.41
M_2**	17.41	145.05	10.76	781.53	74.33	70.51	117.33	85.90

*M_1 = 95% Confidence Interval lower limit
**M_2 = 95% Confidence Interval upper limit

Appendix Table 1e. Raw data for cardiac muscle of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	34.48	66.02	19.75	789.88	170.37	170.69	252.98	91.16
2	36.78	66.76	34.57	799.38	218.29	220.13	290.13	154.53
3	37.91	67.69	27.64	809.46	179.67	180.60	268.63	184.07
4	38.80	80.31	29.03	793.61	186.69	197.42	289.75	66.86
5	32.88	77.26	27.69	810.67	178.07	179.10	253.47	209.15
6	39.22	85.09	26.98	800.77	179.28	178.63	279.39	265.95
7	52.93	75.33	33.52	810.61	218.22	227.41	370.74	225.25
8	43.06	82.49	30.50	807.06	211.32	213.42	300.29	254.78
9	44.47	49.56	26.94	793.64	190.66	189.96	267.38	172.87
10	50.42	88.27	34.63	808.26	227.58	236.60	324.22	288.76
11	45.13	77.95	29.66	813.05	216.30	221.92	340.77	241.86
12	47.59	85.82	27.29	790.23	195.44	205.16	419.86	164.45
13	34.71	65.21	26.09	805.59	166.00	160.29	257.27	191.84
14	44.45	58.73	26.29	788.15	160.45	152.35	227.11	230.67
15	36.35	64.31	24.24	799.87	173.81	202.34	224.08	198.32
16	35.82	71.95	29.22	815.08	200.86	204.82	243.77	95.96
17	39.68	69.04	26.97	803.96	171.60	169.74	314.64	136.24
18	51.54	75.79	24.65	813.27	230.23	239.90	---	125.73
19	47.90	61.78	32.85	798.89	217.09	226.82	427.46	280.08
20	42.16	78.54	28.55	807.35	224.63	240.27	---	240.75
21	58.15	65.16	28.75	798.10	198.76	203.25	416.84	221.39
22	52.47	70.26	33.28	800.85	199.06	201.81	356.25	212.28
23	23.27	58.70	24.71	792.14	136.86	128.58	179.08	150.54
24	20.65	65.29	22.19	812.52	184.27	187.05	300.11	206.06
25	32.86	108.99	28.51	788.33	198.55	216.46	223.84	180.09
26	18.92	131.08	22.01	800.50	205.55	223.68	125.23	220.63
27	41.28	101.62	36.14	808.84	275.91	308.42	331.33	163.16
28	25.38	90.85	24.62	817.29	192.53	208.19	196.38	190.45
29	36.24	110.46	35.77	820.99	257.44	285.15	270.14	185.28
30	41.97	107.79	27.67	811.38	202.79	226.79	291.85	190.39
31	44.56	75.36	28.38	816.95	229.63	250.25	310.37	186.98
32	45.83	91.20	31.85	817.65	233.43	242.23	315.18	206.44
33	38.80	94.12	30.59	805.46	208.88	229.09	282.60	176.23
MEAN	39.52	79.23	28.82	804.53	204.26	212.69	284.21	190.27
M_1*	36.16	72.90	27.41	801.24	193.43	198.89	258.54	171.49
M_2**	42.89	85.56	30.22	807.82	215.10	226.48	309.88	209.05

Appendix Table 1f. Raw data for liver of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	34.14	99.27	26.44	736.55	230.58	247.63	250.49	299.02
2	29.44	127.39	38.51	740.92	243.73	262.37	257.79	410.32
3	35.82	118.19	47.16	763.94	306.57	335.57	255.68	416.48
4	28.21	126.24	34.86	732.18	224.18	244.84	207.76	201.62
5	34.29	114.33	46.87	747.28	301.41	329.35	242.44	512.37
6	40.49	101.17	34.56	770.72	229.65	242.69	288.44	478.59
7	36.74	103.09	30.29	760.02	196.90	208.99	257.34	240.09
8	23.18	125.36	26.73	758.17	255.40	274.46	161.65	457.34
9	34.15	122.92	42.89	739.65	303.54	305.09	205.33	402.18
10	42.08	126.59	46.48	768.95	305.45	332.79	268.92	494.78
11	33.51	129.81	44.17	770.70	322.12	352.39	253.03	503.27
12	28.55	130.87	41.54	758.77	297.49	326.26	251.88	422.04
13	32.32	94.14	43.49	750.52	275.71	299.25	243.26	501.21
14	26.68	109.67	38.97	743.30	237.83	256.11	163.32	579.98
15	27.39	108.56	36.18	750.29	400.42	442.32	169.75	---
16	21.81	127.09	41.79	734.37	287.26	314.26	152.21	---
17	31.57	90.21	38.08	739.46	248.29	269.92	250.33	163.33
18	27.54	104.21	36.56	754.37	341.47	374.46	260.32	148.64
19	34.38	97.02	43.74	750.00	289.05	315.92	306.81	174.56
20	31.42	98.34	42.70	752.07	335.96	369.69	370.99	226.98
21	23.74	90.26	44.51	758.41	307.72	335.66	170.18	290.17
22	48.22	103.69	44.71	794.88	267.42	282.64	286.72	245.84
23	55.56	66.13	49.87	797.25	268.29	296.57	429.58	157.41
24	25.47	98.23	33.73	756.49	279.97	300.34	246.82	202.85
25	39.82	95.23	46.83	770.57	326.14	365.80	244.06	297.55
26	36.77	110.89	39.25	752.62	275.59	307.96	243.37	---
27	45.31	150.16	59.93	760.87	---	---	363.68	345.66
28	49.52	105.49	51.42	755.61	345.70	373.68	361.20	385.70
29	39.18	121.06	46.59	771.78	335.11	377.68	349.06	344.15
30	36.90	144.69	46.27	780.54	332.85	385.33	420.50	331.94
31	39.54	119.36	41.79	762.69	311.20	372.60	272.60	271.49
32	39.07	126.09	42.69	766.10	315.00	349.59	368.75	271.95
33	36.74	117.86	43.60	755.98	311.38	350.78	366.07	347.46
MEAN	34.61	113.20	41.05	756.42	297.10	334.68	328.40	321.75
M_1*	31.94	107.15	38.84	748.06	280.44	314.51	298.34	328.75
M_2**	37.27	119.26	43.26	764.79	313.76	354.85	358.47	314.75

*M_1 = 95% confidence interval I lower limit

**M_2 = 95% confidence interval I upper limit

Appendix Table 1g. Raw data for spleen of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	28.04	94.25	30.79	778.24	263.52	289.52	205.73	145.74
2	41.08	82.03	39.58	791.04	333.28	358.08	327.24	419.31
3	30.15	97.69	40.04	751.93	260.28	281.56	213.64	344.85
4	---	---	44.15	740.76	283.92	---	---	179.02
5	17.27	100.58	30.85	756.65	198.39	210.59	122.10	134.86
6	58.70	78.49	54.56	854.49	386.59	391.37	418.17	537.94
7	36.72	113.95	40.14	805.83	260.93	281.03	257.55	335.28
8	33.31	107.57	37.91	783.83	263.61	281.14	224.63	261.09
9	28.31	107.85	38.75	757.55	271.14	295.72	170.54	249.65
10	34.02	114.09	43.77	780.52	284.36	306.68	217.41	300.27
11	49.99	105.78	51.79	817.66	397.78	430.17	377.42	388.46
12	31.06	109.89	54.24	761.61	343.29	269.85	274.03	175.17
13	27.64	100.28	50.62	765.77	337.00	248.80	167.81	160.94
14	23.87	93.12	37.05	754.47	226.11	140.56	146.12	173.17
15	21.88	101.12	35.84	762.32	374.52	412.54	135.60	154.13
16	22.87	91.28	36.68	777.85	295.13	272.12	164.92	91.15
17	31.18	94.27	39.34	729.41	250.31	270.07	247.4	143.44
18	34.89	100.51	50.81	765.28	287.96	312.46	235.18	141.46
19	27.85	104.12	43.44	738.51	287.07	314.29	248.55	175.34
20	50.71	79.13	54.19	776.68	426.36	471.02	----	269.25
21	65.38	93.65	57.08	767.39	350.35	776.77	---	237.64
22	57.51	102.49	59.22	765.07	237.57	254.42	390.47	---
23	40.11	70.97	44.13	814.10	244.43	257.87	308.68	277.12
24	58.38	81.43	48.61	813.85	403.66	443.49	370.34	384.12
25	24.93	128.17	44.35	729.87	308.87	346.69	169.88	279.35
26	31.56	131.88	51.78	794.63	363.31	409.71	208.88	---
27	17.86	125.59	48.05	778.17	347.60	392.74	---	---
28	29.84	108.89	41.07	750.64	321.17	361.68	231.11	202.39
29	54.60	119.85	49.60	767.84	349.78	394.37	257.92	269.35
30	31.54	128.39	48.81	781.85	357.73	405.49	219.53	225.73
31	35.29	122.57	44.28	781.69	358.28	404.52	245.80	306.62
32	68.97	120.57	46.66	771.73	341.97	380.75	----	154.39
33	48.25	99.32	64.35	818.63	439.42	497.22	351.71	---
MEAN	35.62	103.74	43.05	775.38	305.92	336.41	248.56	234.43
H_1*	30.79	98.09	40.26	766.31	283.71	309.39	216.47	202.01
H_2**	41.44	109.39	45.84	784.46	328.13	363.44	280.66	266.84

Appendix Table 1h. Raw data for gut of rainbow trout acclimated to 10°C.

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PEG}$ (ml/kg)
1	44.56	54.74	34.17	807.18	298.23	315.44	326.94	268.95
2	44.75	54.08	33.29	823.81	---	200.14	391.86	---
3	63.36	62.76	62.61	821.99	406.99	444.72	448.97	431.45
4	58.11	60.67	55.20	813.77	354.98	387.59	427.96	---
5	57.78	67.64	61.22	806.24	393.69	429.72	408.51	476.17
6	68.16	64.34	62.61	793.58	410.04	453.72	485.56	500.87
7	73.84	60.88	63.79	828.65	414.67	478.94	517.20	495.02
8	62.02	66.48	58.45	801.08	406.44	441.67	432.52	512.65
9	69.47	61.67	64.83	793.28	458.81	505.75	417.70	359.89
10	75.81	72.13	69.39	801.89	456.01	501.07	484.48	507.70
11	82.29	56.54	71.29	810.01	519.90	573.49	----	452.66
12	69.97	67.16	57.99	808.11	413.87	454.18	---	382.67
13	71.19	52.45	69.28	806.59	440.81	481.98	527.66	426.97
14	60.24	52.76	72.35	789.87	441.55	484.39	491.81	419.37
15	----	----	----	----	----	----	----	460.15
16	66.41	42.34	68.02	802.11	467.56	512.24	478.68	302.69
17	67.82	58.09	60.98	812.42	387.99	421.49	537.72	439.4
18	73.89	58.90	61.62	802.99	575.53	637.78	698.17	425.08
19	77.25	52.55	68.67	804.81	452.79	499.44	640.38	514.90
20	72.61	45.72	67.79	817.47	533.36	589.98	587.34	425.81
21	77.85	53.21	57.59	794.89	398.15	434.60	528.06	522.33
22	53.24	56.37	60.73	802.52	399.13	434.27	361.48	428.86
23	43.06	39.74	61.13	819.46	338.59	359.70	341.38	415.66
24	42.25	50.42	53.64	807.92	445.43	488.34	497.88	480.49
25	67.51	104.26	69.25	777.63	457.97	518.87	460.02	378.59
26	63.58	103.92	63.31	796.34	443.51	501.80	419.49	383.65
27	65.20	85.31	66.98	795.91	484.54	549.62	523.32	404.07
28	72.05	73.33	53.29	811.70	416.73	469.88	557.89	403.65
29	73.40	89.80	50.60	801.31	478.95	484.57	547.15	338.44
30	67.88	86.43	65.66	795.52	478.82	541.32	472.93	---
31	74.51	78.97	55.95	802.69	457.71	512.16	518.98	463.85
32	---	79.77	---	787.69	---	---	---	326.33
33	69.27	81.51	68.15	804.73	465.36	526.95	502.49	389.12
MEAN	66.28	69.65	61.39	804.67	444.99	472.16	498.62	419.89
H_1*	62.40	59.34	58.02	799.35	415.42	461.97	469.10	396.66
H_2**	70.16	71.46	64.59	666.10	474.57	524.36	527.13	443.74

*H_1 = 95% Confidence interval lower limit
**H_2 = 95% Confidence interval upper limit

Appendix Table 11. Raw data for brain of rainbow trout acclimated to 10°C.

FISH #	Na^+ (ml/kg)	K^+ (ml/kg)	Cl^- (ml/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	$H_2O^{ecs}_{PSG}$ (ml/kg)
1	60.94	60.96	30.13	796.49	262.76	276.26	447.23	17.41
2	---	---	---	---	---	---	---	74.79
3	70.39	61.02	55.76	803.90	349.47	328.53	595.42	150.37
4	61.78	80.92	38.85	794.59	249.84	269.89	454.98	16.11
5	72.06	63.61	45.12	805.83	300.16	306.13	509.40	106.96
6	58.77	82.09	38.52	791.92	262.61	277.08	418.67	80.83
7	66.50	76.18	43.46	850.75	282.51	301.62	486.80	65.93
8	72.98	75.76	43.57	807.43	303.97	320.86	5 8.95	83.26
9	63.46	53.40	39.71	807.41	304.29	430.07	501.82	93.12
10	71.95	87.52	47.15	800.10	276.99	294.94	459.81	43.07
11	77.57	78,61	40.49	813.52	285.28	315.08	585.72	61.94
12	72.19	69.70	53.19	794.84	257.69	251.86	650.89	72.54
13	80.25	47.35	43.36	796.63	275.89	285.52	594.81	84.89
14	74.88	50.72	44.25	815.97	270.05	278.26	458.39	118.48
15	52.75	53.15	32.55	800.92	415.58	456.21	326.92	179.86
16	67.16	54.96	39.72	764.98	273.88	289.01	484.08	34.21
17	78.37	65.65	46.75	792.04	287.45	318.11	699.35	42.49
18	70.26	65.31	41.76	821.72	390.22	424.57	683.87	29.93
19	74.89	72.61	48.47	798.23	340.31	348.53	668.32	76.26
20	66.42	68.61	41.76	803.24	303.56	358.47	---	27.88
21	58.38	70/99	37.95	812.16	262.39	278.91	418.49	32.13
22	56.25	76.19	38.41	793.68	239.74	239.09	402.28	20.92
23	92.59	102.61	44.34	801.39	245.59	264.48	---	26.88
24	52.21	68.16	38.08	814.81	316.05	340.37	505.95	83.32
25	47.78	104.74	51.65	800.81	359.71	404.38	325.58	94.72
26	78.48	103.68	54.18	808.28	380.15	426.01	519.43	78.80
27	74.79	93.54	55.06	803.73	398.31	450.07	600.29	54.65
28	63.33	93.49	41.69	813.61	328.01	364.86	490.51	39.84
29	62.71	112.43	45.43	795.78	326.96	367.05	474.92	35.71
30	63.99	110.88	47.37	805.61	346.44	392.16	444.98	41.32
31	76.61	90.12	51.72	799.82	418.48	472.96	533.61	81.14
32	75.82	87.16	47.38	807.92	350.91	385.82	521.53	26.83
33	65.08	75.57	59.23	796.36	404.45	456.26	473.04	48.70
MEAN	69.29	77.30	44.45	802.97	316.91	344.63	503.29	63.31
M_1*	65.73	70.83	42.02	798.88	296.68	319.80	472.08	50.02
M_2**	72.87	83.77	46.89	807.05	337.14	369.45	534.52	76.60

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix V

Appendix Table I. Raw data for rainbow trout acclimated to 2°C (summer series).

FISH No.	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
6	M	23.5	153.5	187.0	3.06	136.72	930.90
7	M	23.5	149.8	165.2	1.19	134.49	945.00
12	M	22.5	132.8	155.9	3.23	131.45	951.77
13	F	22.5	140.5	168.2	1.19	134.94	149.77
14	M	26.5	196.5	160.9	0.89	142.55	978.13
15	M	23.5	145.1	152.8	3.92	123.35	965.63
22	F	25.5	190.1	159.0	1.22	132.62	965.74
23	F	24.5	171.9	---	1.19	130.97	948.63
24	F	23.0	124.9	154.7	1.36	128.98	961.11
25	F	23.5	153.5	154.7	0.98	130.14	933.33
26	M	26.0	200.0	156.4	1.41	131.63	910.18
27	F	22.0	117.5	156.5	1.53	131.96	914.69
28	-	16.0	40.1	---	---	125.01	----
29	-	15.5	38.1	165.2	--	103.83	----
30	-	15.0	35.6	154.1	2.04	126.99	919.61
31	F	24.0	163.5	151.9	4.26	126.69	942.51
32	F	23.0	133.0	155.9	2.04	125.70	940.60
MEAN		22.4	134.5	159.89	1.98	129.44	944.38
M_1		20.6	107.9	154.94	1.35	125.16	933.66
M_2		24.2	161.2	164.85	2.58	133.72	955.10

Appendix Table I b. Raw data for rainbow trout acclimated to 2°C (summer series). Data is for Post-opercular Muscle.

FISH No.	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
6	---	123.03	5.93	785.90	29.29	--	162.48	2.54
7	---	147.29	6.28	787.40	43.05	--	197.61	0.66
12	9.69	128.47	6.18	789.12	32.86	6.04	169.74	2.46
13	6.86	102.37	6.28	799.22	40.54	0.05	134.87	1.07
14	8.29	108.98	--	787.11	---	--	----	--
15	8.05	102.35	--	789.44	---	--	----	--
22	14.43	122.95	6.53	784.68	45.03	9.83	166.15	0.82
23	10.59	140.98	5.56	786.41	38.44	--	188.42	0.70
24	12.68	---	6.43	788.19	---	--	----	--
25	10.39	108.16	7.21	789.94	51.39	3.31	146.38	0.71
26	10.06	112.63	7.17	790.12	49.87	3.05	152.04	0.82
27	10.79	105.09	6.92	788.59	43.14	5.01	141.26	1.29
28	13.94	113.96	5.83	790.78	---	--	----	--
29	10.08	102.40	6.54	787.25	---	--	----	--
30	8.97	98.97	6.84	790.69	42.90	3.15	132.23	1.86
31	10.40	119.13	6.88	779.79	34.55	6.92	159.86	3.36
32	9.76	125.79	6.07	786.31	39.84	4.75	168.40	1.43
MEAN	10.33	116.42	6.45	788.26	41.08	4.68	159.97	1.48
M_1*	9.19	108.82	6.18	786.22	36.87	2.54	147.19	0.91
M_2**	11.46	124.01	6.72	790.30	45.28	6.82	172.75	2.04

*M_1 - 95% Confidence Interval lower limit

** M_2 - 95% Confidence Interval upper limit

Appendix Table 1 c. Raw data for mid-dermal muscle of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	17.87	130.29	4.74	783.50	21.23	16.24	173.46	2.41
7	---	141.86	5.11	783.70	33.80	---	189.14	0.74
12	14.03	129.37	5.69	785.62	28.24	12.71	161.44	2.61
13	7.70	102.09	5.41	777.32	34.21	2.62	137.33	1.07
14	6.34	118.13	--	787.09	---	---	---	--
15	7.58	117.22	--	788.38	---	---	---	--
22	13.59	132.11	4.78	781.92	31.38	11.46	175.97	0.82
23	8.85	143.47	4.54	786.02	30.43	---	189.33	0.73
24	12.32	---	5.56	788.86	---	---	---	--
25	9.39	108.47	5.70	785.90	39.49	4.39	145.27	0.75
26	9.62	115.83	6.08	784.04	40.13	4.56	155.63	1.07
27	11.09	94.92	6.74	783.31	42.90	5.91	128.11	1.46
28	11.14	103.00	6.07	787.76	---	---	---	--
29	8.33	105.66	5.49	780.47	---	---	---	--
30	5.95	103.46	8.39	788.08	56.42	3.75	141.25	1.67
31	7.56	118.04	5.39	777.38	21.68	5.65	156.08	3.49
32	7.81	124.84	6.18	783.16	40.77	1.96	168.05	1.42
MEAN	9.95	117.74	5.72	784.38	35.06	6.75	160.09	1.52
M_1*	8.24	110.	5.20	782.52	28.86	2.71	147.46	0.96
M_2**	11.66	125.46	6.24	786.24	41.27	10.79	172.72	2.08

Appendix Table 1 d. Raw data for caudal muscle of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	---	133.22	7.30	785.60	41.14	---	178.78	2.25
7	---	126.14	7.86	783.40	54.50	---	172.97	0.73
12	14.33	114.50	7.97	787.95	45.56	9.74	154.03	2.67
13	6.10	105.06	8.70	783.99	59.69	---	144.95	0.89
14	9.82	94.99	--	787.09	---	---	---	--
15	9.76	108.28	--	792.76	---	---	---	--
22	10.73	145.01	11.49	777.46	84.39	4.78	209.08	0.43
23	10.71	143.20	5.75	791.39	39.96	---	190.51	0.69
24	14.04	---	8.99	790.36	---	---	---	--
25	12.25	127.28	9.96	790.46	74.14	1.09	177.58	0.44
26	9.65	110.86	8.78	789.21	61.12	0.13	152.14	1.01
27	14.54	104.58	8.81	785.24	60.14	7.07	144.10	1.21
28	16.40	115.47	11.89	786 36	---	---	---	--
29	12.66	117.05	11.69	781.73	---	---	---	--
30	10.11	102.01	9.20	796.54	62.72	0.61	138.84	1.68
31	10.95	123.65	9.30	782.12	55.83	3.40	169.92	3.07
32	10.75	130.42	7.85	785.51	55.07	2.96	178.39	1.27
MEAN	11.92	118.86	9.04	786.89	57.86	3.72	167.61	1.36
M_1*	10.33	111.05	8.11	784.50	49.76	0.92	154.15	0.80
M_2**	13.52	126.66	9.97	789.28	65.95	6.53	181.07	1.92

Appendix Table 1 e. Raw data for cardiac muscle of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	76.96	72.62	40.14	819.70	264.20	49.66	129.27	7.23
7	75.10	66.99	42.71	819.10	285.80	62.78	124.98	8.01
12	71.66	92.03	45.44	829.11	311.10	44.75	175.82	8.78
13	49.68	61.68	42.83	819.77	285.70	---	114.85	8.01
14	51.83	35.66	---	826.41	---	---	---	--
15	42.44	40.37	---	792.68	---	---	---	--
22	72.27	---	41.56	809.52	282.00	52.00	---	7.89
23	54.68	85.85	36.26	811.59	249.50	---	148.57	6.44
24	68.84	---	40.75	822.78	284.30	46.17	---	7.58
25	53.72	52.79	44.36	804.23	306.80	12.58	105.52	8.91
26	60.96	67.20	51.44	815.11	351.70	12.85	135.42	10.94
27	54.59	40.17	62.47	816.79	289.60	17.59	75.36	8.07
28	64.15	63.05	51.81	804.88	373.00	---	---	11.99
29	60.66	71.78	51.55	800.52	---	---	---	---
30	62.76	47.29	46.08	829.27	326.60	15.06	97.75	9.16
31	60.44	64.20	39.75	802.69	283.40	10.55	119.93	7.56
32	57.34	51.49	43.96	811.09	314.70	9.61	103.31	8.86
MEAN	58.32	60.54	46.07	818.27	300.51	30.79	120.44	8.53
M_1*	54.49	51.41	41.58	809.20	281.85	16.52	101.96	7.69
M_2**	62.15	69.68	50.56	827.34	319.17	45.06	138.91	9.37

* M_1 = 95% confidence limit for mean (lower limit)
** M_2 = 95% confidence limit for mean (upper limit)

Appendix Table 1 f. Raw data for liver of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	36.66	108.14	42.49	755.40	195.39	---	192.04	28.17
7	---	111.23	40.65	744.40	---	---	---	---
12	24.87	95.42	38.94	745.59	161.18	---	162.94	30.48
13	29.50	44.56	40.89	750.97	176.83	---	77.25	29.66
14	29.9	62.92	---	757.02	188.33	---	110.35	---
15	29.41	74.92	---	749.92	197.30	---	141.87	---
22	42.64	---	41.80	762.56	274.93	---	---	10.95
23	29.98	93.31	41.30	752.86	---	---	---	---
24	31.24	116.79	44.09	749.52	206.04	---	214.38	32.23
25	40.11	44.38	45.88	766.14	256.89	---	86.65	24.44
26	24.38	83.60	46.82	760.38	150.62	---	136.75	44.27
27	33.60	54.91	42.84	747.47	208.47	---	101.28	28.44
28	36.87	37.02	38.65	743.34	---	---	---	---
29	20.78	67.92	45.87	751.66	---	---	---	---
30	23.53	37.71	39.08	759.38	149.06	---	61.29	33.02
31	31.88	76.04	47.99	749.68	209.99	---	139.24	39.63
32	28.46	75.44	45.82	757.52	182.28	---	130.49	39.82
MEAN	30.82	73.96	42.87	753.05	196.72	---	129.54	31.01
M_1*	27.59	60.05	41.18	749.57	174.33	---	100.54	25.02
M_2**	34.04	87.87	44.56	756.53	219.11	---	158.55	37.00

A

Appendix Table 1 g. Raw data for gut of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	63.96	66.08	33.29	801.40	219.10	---	112.33	5.73
7	---	81.83	34.52	798.70	231.00	---	143.66	6.08
12	41.93	45.80	35.80	819.44	245.10	---	78.37	6.24
13	---	---	27.56	773.03	183.80	---	---	4.65
14	25.97	---	---	778.88	---	---	---	---
15	24.23	23.54	---	782.93	---	---	---	---
22	39.84	---	37.59	795.38	255.10	---	---	6.96
23	32.87	54.52	27.52	800.26	189.10	---	88.33	4.51
24	44.12	80.75	39.82	768.43	277.80	---	163.81	8.13
25	26.36	34.31	37.34	784.09	258.20	---	64.76	7.11
26	31.66	35.56	43.64	781.09	298.40	---	72.79	9.04
27	47.09	37.03	43.20	773.03	294.60	---	76.46	9.04
28	36.40	53.67	37.48	791.67	269.80	---	---	7.19
29	34.18	49.45	24.41	792.61	211.60	---	---	4.19
30	38.62	83.06	27.71	772.91	196.40	---	143.36	4.80
31	46.60	45.80	30.72	---	---	---	---	---
32	56.76	52.79	37.13	755.00	265.80	---	106.80	7.60
MEAN	39.37	53.16	34.51	785.55	242.56	---	109.60	6.52
M_1*	33.16	42.33	31.26	777.13	220.57	---	83.66	5.59
M_2**	45.59	63.98	37.75	793.97	264.54	---	135.55	7.46

Appendix Table 1 h. Raw data for brain of rainbow trout acclimated to 2°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
6	---	87.99	38.78	795.30	255.30	---	161.49	7.18
7	99.95	83.50	38.97	791.00	260.80	---	156.90	7.35
12	73.81	75.23	48.99	822.78	335.40	44.16	152.34	10.06
13	52.96	65.76	41.92	795.03	279.60	11.51	126.94	8.13
14	70.68	53.59	---	817.78	---	---	---	---
15	61.00	52.33	---	813.72	---	---	---	---
22	95.60	---	43.31	815.79	293.20	93.64	---	8.30
23	72.94	109.14	42.62	817.31	292.90	---	207.45	8.12
2	70.56	---	36.96	807.91	257.90	55.79	---	6.72
25	64.91	47.06	55.19	821.59	381.70	13.33	105.13	12.54
26	76.32	47.96	---	805.56	---	---	---	---
27	65.86	47.19	46.83	801.52	319.40	28.77	96.86	9.71
28	---	---	---	---	---	---	---	---
29	---	---	---	---	---	---	---	---
30	51.33	89.67	49.11	816.67	348.60	---	189.81	10.49
31	72.72	82.38	43.15	809.78	306.30	50.09	161.29	8.59
32	64.82	59.99	39.62	796.61	279.40	41.17	114.89	7.54
MEAN	70.27	69.38	43.74	808.52	301.90	40.25	147.41	8.77
M_1*	62.94	57.25	40.52	800.73	276.01	34.13	131.19	7.86
M_2**	77.61	81.50	47.15	816.31	327.79	46.38	163.63	9.68

* M_1 = 95% Confidence Interval Lower Limit

** M_2 = 95% Confidence Interval Upper Limit

Appendix Table 2 a. Raw data for rainbow trout acclimated to 10°C (summer series) - physical characteristics and plasma electrolyte and water content.

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
4	F	25.0	183.0	162.10	1.69	135.92	962.90
5	F	25.5	181.0	156.40	1.32	131.14	963.40
10	M	26.5	203.0	174.10	1.46	134.09	970.77
11	M	25.0	165.1	161.10	1.44	131.45	966.94
16	F	27.0	218.0	152.80	1.05	128.65	943.06
17	F	25.5	173.0	156.40	1.79	131.46	944.06
18	F	23.5	142.1	162.30	2.26	130.14	970.22
19	F	26.5	220.1	160.30	1.19	131.96	958.14
20	F	25.0	186.5	137.90	5.66	98.54	963.13
21	M	25.0	162.9	154.70	2.29	109.45	968.57
36	F	23.5	129.6	157.20	1.61	124.71	937.17
37	F	27.0	205.7	146.60	2.04	129.35	956.52
38	F	25.5	184.5	154.70	2.15	131.00	933.53
41	M	25.0	179.2	156.40	0.80	132.99	904.98
42	-	15.0	29.2	---	--	127.36	---
43	-	14.5	34.5	155.30	1.82	130.34	---
44	-	12.5	17.9	---	--	131.33	---
MEAN		23.2	150.2	156.22	1.90	127.64	953.08
M_1*		20.7	116.3	151.34	1.28	122.79	942.32
M_2**		25.6	184.1	161.09	2.53	132.49	963.84

Appendix Table 2 b. Raw data for post-opercular muscle of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
4	16.40	126.94	5.93	796.00	36.51	13.80	167.06	1.27
5	---	123.13	7.23	794.90	50.12	---	165.24	0.88
10	13.11	127.36	7.40	790.12	49.82	5.99	171.94	0.97
11	10.60	138.22	7.24	783.93	50.43	3.38	188.34	0.63
16	15.63	121.80	7.66	770.57	56.28	9.64	170.44	0.59
17	11.91	124.63	8.33	786.12	56.75	4.16	170.75	1.19
18	13.36	119.93	7.54	765.62	48.43	7.46	162.54	1.68
19	15.04	137.85	8.23	798.34	58.85	8.96	186.55	0.65
20	16.01	106.63	7.08	809.64	40.15	13.87	138.28	4.10
21	15.78	119.14	--	811.80	---	---	---	---
36	12.39	109.31	8.34	785.38	60.23	4.03	150.61	1.14
37	12.10	129.56	7.56	785.96	50.79	6.33	176.09	1.35
38	11.77	138.93	7.46	783.44	49.53	5.59	189.16	1.32
41	12.87	103.67	9.29	799.19	67.14	3.24	141.54	0.49
42	12.71	108.06	7.34	788.41	---	--	---	--
43	9.53	131.09	7.40	792.45	49.99	2.38	176.44	1.19
44	10.09	140.26	8.75	803.74	---	--	---	--
MEAN	13.14	123.90	7.67	791.90	51.80	6.85	167.99	1.26
M_1*	11.96	117.86	7.26	786.70	47.30	4.55	158.97	0.75
M_2**	14.33	129.94	8.09	797.30	56.30	9.15	177.02	1.76

Appendix Table 2 c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
4	15.85	133.79	5.21	792.80	31.47	14.12	175.66	1.22
5	15.70	126.96	5.64	786.10	37.94	13.19	174.16	0.89
10	12.47	---	6.23	787.00	---	---	---	--
11	11.21	134.76	5.92	780.49	39.88	6.46	181.88	0.92
16	9.69	129.19	6.71	771.81	48.68	3.82	176.63	0.58
17	9.06	129.62	6.45	784.91	42.14	3.32	174.41	1.23
18	11.63	125.19	7.10	765.78	45.43	5.75	170.31	1.60
19	13.32	132.27	6.74	792.86	46.97	7.76	177.27	0.73
20	12.38	---	5.16	---	---	---	---	--
21	10.41	124.59	--	804.56	---	--	---	--
36	9.19	116.36	6.09	781.84	41.94	3.51	157.04	1.16
37	9.89	121.18	6.64	779.59	42.85	4.89	164.36	1.49
38	10.88	136.85	6.22	773.75	39.25	6.49	171.18	1.53
41	11.19	125.73	7.74	799.51	55.79	3.51	166.54	0.43
42	11.59	116.43	6.17	786.82	---	---	---	--
43	6.77	130.54	6.15	786.79	40.68	3.41	174.36	1.25
44	11.87	114.31	7.59	803.84	---	--	---	--
MEAN	11.47	125.91	6.36	787.90	43.71	6.87	173.13	1.09
M_1*	10.41	120.33	5.97	783.79	38.89	5.86	167.98	0.85
M_2**	12.53	131.49	6.75	792.02	48.53	8.08	178.49	1.33

M_1* = 95% Confidence interval lower limit
M_2** = 95% Confidence interval upper limit

Appendix Table 2 d. Raw data for caudal muscle of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
4	19.54	121.05	7.75	795.40	50.13	15.52	162.31	1.26
5	---	120.16	8.68	789.80	61.57	---	164.89	0.83
10	16.81	117.99	9.39	795.07	65.27	7.48	175.74	0.88
11	20.70	133.60	8.97	789.58	63.43	14.43	183.98	0.80
16	10.76	119.75	8.12	776.64	59.82	2.26	166.97	0.59
17	10.21	115.81	9.10	785.97	62.11	0.69	157.07	1.29
18	13.84	115.98	8.76	790.77	57.79	6.09	158.05	1.69
19	12.07	96.98	8.43	795.48	58.51	4.91	131.46	0.99
20	18.07	104.48	7.34	---	---	---	---	---
21	11.67	152.11	11.82	808.86	---	---	---	---
36	14.30	119.39	9.01	782.36	66.62	5.35	166.66	0.98
37	15.55	135.27	8.26	789.04	56.65	9.89	181.81	1.27
38	11.88	---	6.94	783.42	---	---	---	---
41	12.15	113.28	9.11	798.22	66.10	2.47	153.29	0.44
42	11.99	106.16	7.72	787.23	---	---	---	---
43	9.51	105.06	8.00	794.74	52.70	1.79	141.45	1.52
44	11.94	110.04	11.76	806.67	---	---	---	---
MEAN	13.87	117.01	8.77	791.70	60.04	6.43	161.97	1.05
M_1*	12.07	111.13	8.09	787.24	56.74	3.08	152.22	0.81
M_2**	15.66	122.89	9.45	796.16	64.02	9.77	171.72	1.28

Appendix Table 2 e. Raw data for cardiac muscle of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
4	---	62.03	46.06	812.90	304.90	---	121.09	9.09
5	81.24	65.17	42.27	797.70	290.00	70.69	127.61	8.35
10	71.42	84.24	45.97	815.53	308.50	34.93	165.26	9.08
11	56.34	44.30	41.91	818.79	286.90	19.02	82.51	7.89
16	48.54	4.53	39.25	796.46	274.60	12.61	84.78	7.51
17	63.87	66.96	44.54	803.79	304.90	32.44	133.12	8,94
18	61.61	40.37	37.73	817.07	260.90	34.64	71.52	6.79
19	62.59	---	42.31	808.89	288.60	31.38	---	8.12
20	58.01	68.43	33.98	782.61	310.30	35.51	141.17	7.21
21	65.74	62.03	41.78	824.56	343.50	26.19	127.31	8.69
36	55.96	44.59	40.38	810.81	291.30	19.87	84.93	7.79
37	62.81	97.01	45.36	828.95	315.60	32.22	187.72	8.84
38	50.65	80.74	44.49	804.88	303.60	7.35	159.78	9.41
41	47.74	55.75	38.93	811.52	263.40	11.94	101.33	7.12
42	32.55	52.36	40.16	810.81	283.80	---	---	7.62
43	34.75	63.05	40.49	829.27	279.60	15.77	113.78	7.36
44	34.08	80.38	42.85	812.50	293.60	---	---	8.27
MEAN	55.49	61.71	41.67	811.00	294.40	27.47	121.57	8.12
M_1*	48.23	53.33	40.03	804.90	283.95	18.36	101.61	7.71
M_2**	62.76	70.08	43.31	817.10	304.80	36.58	141.52	8.53

Appendix Table 2 f. Raw data for liver of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
4	35.07	106.41	50.65	761.60	221.15	---	196.19	38.10
5	45.92	106.94	54.75	751.80	300.28	---	235.97	54.04
10	43.30	111.97	55.07	762.22	268.14	---	225.83	38.69
11	35.39	94.91	52.21	754.47	225.49	---	178.81	42.67
16	35.22	84.22	51.93	787.82	230. 6	---	163.41	43.02
17	40.58	87.89	52.76	778.28	260.03	---	178.97	39.00
18	32.95	93.34	55.32	756.71	209.10	---	169.59	51.33
19	41.57	108.63	48.33	760.59	263.77	---	217.14	27.11
20	34.30	93.19	---	---	---	---	---	---
21	30.77	97.06	44.18	772.84	204.51	---	169.96	28.35
36	32.97	---	50.45	729.19	202.96	---	---	46.88
37	45.49	91.09	60.96	777.48	301.25	---	189.97	46.19
38	39.13	93.59	55.44	768.14	250.55	---	185.83	44.21
41	44.38	67.75	24.62	772.98	272.61	---	134.16	41.25
42	32.83	71.82	43.91	740.56	---	---	---	---
43	31.86	81.70	51.92	755.09	---	---	---	---
44	22.13	80.01	58.67	751.59	---	---	---	---
MEAN	36.99	92.35	50.66	757.65	248.70	---	187.07	40.76
M_1*	34.09	85.45	50.11	751.97	229.19	---	168.86	37.03
M_2**	39.89	99.24	55.22	763.33	267.30	---	205.27	44.48

M_1* = 95% Confidence Interval lower limit
M_2** = 95% Confidence Interval upper limit

Appendix Table 2 g. Raw data for gut of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}Cl$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
4	63.48	71.95	31.03	77.50	205.50	---	126.06	5.46
5	64.24	72.17	47.62	790.90	326.80	---	154.58	10.26
10	59.31	70.31	45.88	804.85	307.90	---	140.58	9.24
11	48.27	72.30	42.88	808.22	293.60	---	139.67	8.33
16	34.52	65.23	49.73	748.95	347.90	---	161.74	12.39
17	30.98	50.04	40.89	768.08	279.90	---	101.47	8.38
18	46.71	40.84	45.12	760.39	312.00	---	89.51	10.07
19	57.81	---	42.85	758.62	292.20	---	---	9.20
20	47.50	---	29.12	778.29	265.90	---	---	5.69
21	44.21	87.10	35.37	776.59	290.80	---	177.92	7.29
36	42.35	29.38	38.57	769.45	278.20	---	58.89	7.89
37	38.28	53.71	47.61	814.80	331.30	---	120.03	9.84
38	36.69	91.99	46.24	795.43	315.50	---	190.27	10.23
41	38.19	62.26	49.82	808.98	337.10	---	131.37	10.57
42	21.16	41.61	27.73	774.19	195.90	---	---	4.81
43	29.82	32.98	28.13	779.66	194.20	---	55.73	4.81
44	---	55.33	26.55	771.43	181.90	---	---	4.51
MEAN	43.97	60.13	39.71	782.57	279.75	---	126.76	8.18
M_1*	37.29	49.85	35.41	772.50	252.16	---	101.62	6.95
M_2**	50.66	70.42	44.01	792.64	307.34	---	151.89	9.40

Appendix Table 2 h. Raw data for brain of rainbow trout acclimated to 10°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}Cl$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
4	84.39	70.26	45.21	807.90	299.30	70.53	137.15	8.91
5	87.04	70.00	43.54	800.00	298.80	80.42	138.88	8.69
10	78.00	84.34	---	---	---	---	---	---
11	70.60	55.97	45.96	796.51	314.70	41.31	115.23	9.53
16	---	47.44	40.25	804.76	281.60	---	149.88	7.69
17	77.99	72.83	44.38	797.87	303.80	61.68	156.43	8.99
18	66.76	66.53	47.21	800.90	326.50	29.02	138.75	9.95
19	94.33	45.88	45.76	803.42	312.90	90.05	92.77	9.11
20	71.93	69.97	34.77	801.32	317.60	61.44	140.93	7.18
21	76.40	67.38	39.36	808.92	323.60	54.27	137.31	8.12
36	81.05	52.14	53.15	796.05	383.40	50.36	124.86	12.93
37	76.70	62.12	47.61	815.03	331.30	58.16	127.02	9.83
38	77.59	69.33	43.63	807.29	297.70	61.88	134.81	9.09
41	69.94	77.10	47.18	813.95	319.30	40.44	155.35	9.55
42	---	---	---	---	---	---	---	---
43	---	---	---	---	---	---	---	---
44	---	---	---	---	---	---	---	---
MEAN	77.88	65.45	44.46	804.20	316.20	58.29	134.57	9.19
M_1*	73.29	58.82	41.74	801.50	301.40	47.42	124.24	8.36
M_2**	82.46	72.08	47.19	806.90	331.01	69.18	144.89	10.03

* M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 3 a. Raw data for rainbow trout acclimated to 18°C (summer series) - physical characteristics and plasma electrolytes and water content

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
2	F	24.0	157.5	168.40	2.45	135.21	952.80
3	M	24.0	167.5	153.90	--	138.43	936.30
8	M	24.0	146.8	157.20	0.85	137.49	967.02
9	-	19.0	88.5	161.50	3.05	123.52	921.76
33	F	22.5	116.5	157.80	1.39	131.53	903.52
34	M	25.5	183.0	154.70	2.35	131.33	913.08
35	F	24.5	158.1	152.20	2.04	128.68	968.85
39	F	24.5	145.5	150.90	2.89	132.16	924.80
40	F	21.0	100.0	---	---	---	---
45	-	15.5	58.6	150.00	1.44	126.47	929.75
46	-	15.5	58.5	165.20	1.95	132.05	954.65
47	-	16.5	59.8	165.20	2.12	124.50=	962.25
48	F	23.0	122.5	155.80	2.71	122.20	967.05
49	M	25.0	155.5	149.70	1.34	131.72	913.26
50	F	23.5	128.5	149.97	2.12	128.77	914.79
51	F	22.0	121.5	155.90	2.47	131.06	936.51
52	M	24.0	142.5	147.60	1.53	135.33	938.65
53	-	--	--	150.60	3.13	131.06	934.78
MEAN		22.2	124.4	156.80	2.13	129.49	939.90
M_1*		20.5	100.5	151.50	1.76	127.56	927.80
M_2**		23.9	148.2	162.10	2.49	131.42	952.10

App
Appendix Table 3 b. Raw data for post-opercular muscle of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
2	19.16	101.06	7.99	781.60	46.58	15.39	137.34	2.30
3	---	115.95	6.23	782.80	---	---	---	--
8	13.90	109.77	7.12	783.23	52.81	9.11	150.22	0.53
9	16.74	112.54	7.50	780.97	45.68	12.73	152.65	2.52
33	11.13	150.04	8.26	804.78	58.91	2.46	210.05	0.70
34	12.66	103.17	8.08	777.61	50.18	6.73	141.67	2.05
35	10.51	112.62	7.47	788.84	48.89	4.15	152.06	1.59
39	12.37	146.59	8.74	801.49	56.06	5.25	196.43	1.79
40	11.79	149.00	7.46	787.15	---	--	---	--
45	11.03	---	--	782.71	---	--	---	--
46	10.81	136.17	7.88	795.32	52.71	2.83	183.23	1.24
47	7.35	149.49	7.58	786.78	54.24	2.19	203.91	1.13
48	12.49	129.05	8.03	792.45	55.14	5.29	174.83	1.75
49	13.65	109.44	7.25	793.06	49.15	8.46	147.03	1.04
50	15.84	118.86	7.16	799.52	46.17	11.83	157.65	1.61
51	10.81	126.30	6.07	780.42	35.91	7.00	169.52	1.83
52	11.18	134.76	6.64	796.28	43.39	6.34	178.90	1.02
53	10.52	146.64	7.59	783.01	47.01	4.67	199.04	1.94
MEAN	12.47	126.55	7.47	788.80	49.50	6.82	169.72	1.54
M_1*	11.04	117.57	7.11	784.80	46.31	4.52	156.91	1.22
M_2**	13.89	135.54	7.83	792.80	52.73	9.11	182.53	1.86

Appendix Table 3 c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
2	--	107.96	6.85	779.60	38.64	--	145.58	2.19
3	--	120.94	5.49	781.20	---	--	---	--
8	13.87	109.77	7.12	783.23	52.81	9.07	150.20	0.53
9	14.80	126.12	5.50	779.64	30.32	13.22	168.18	2.34
33	10.46	135.49	8.13	798.43	57.39	1.89	182.73	0.80
34	10.99	101.79	7.34	776.65	44.06	5.69	138.80	2.12
35	9.95	103.42	7.56	787.09	48.72	3.43	139.93	1.75
39	12.18	149.47	7.51	801.92	46.45	6.84	197.67	1.81
40	9.14	147.24	6.70	782.46	---	--	---	--
45	5.92	150.07	6.72	785.45	48.86	2.51	203.64	0.73
46	9.77	133.34	6.95	796.54	45.12	3.68	177.38	1.32
47	6.69	118.53	6.92	786.74	46.92	1.49	160.08	1.52
48	11.29	130.87	6.03	789.10	36.63	7.14	174.11	1.83
49	13.51	109.19	6.02	789.54	33.71	10.82	146.88	1.12
50	14.31	119.35	6.13	796.76	36.34	11.59	158.55	1.64
51	8.48	125.96	4.99	781.13	26.59	5.74	165.67	1.94
52	10.72	130.28	6.52	796.89	40.44	6.28	183.67	1.09
53	8.66	134.31	5.73	778.39	30.04	5.35	179.56	2.14
MEAN	10.58	125.52	6.56	787.30	40.90	5.95	166.66	1.56
M_1*	9.28	112.76	6.14	784.40	32.09	3.78	155.91	1.25
M_2**	12.07	134.28	6.98	791.10	47.80	8.11	177.41	1.86

*M_1 = 95% confidence interval lower limit
**M_2 = 95% confidence interval upper limit

Appendix Table 3 d. Raw data for cardiac muscle of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
2	---	98.56	8.63	782.10	51.24	-----	134.68	2.33
3	23.66	106.35	7.96	779.90	---	-----	---	---
8	19.61	91.90	10.28	793.19	77.55	12.41	128.50	0.55
9	---	111.08	8.39	780.89	57.50	---	153.28	2.47
33	14.36	144.79	10.41	799.08	75.80	3.32	200.04	0.63
34	13.92	112.61	10.39	77 .18	99.83	4.39	158.52	1.72
35	11.95	111.49	10.15	788.93	70.72	1.65	155.03	1.46
39	13.92	128.73	10.51	805.51	68.44	4.19	174.38	1.99
40	11.75	153.78	8.16	787.31	---	---	---	---
45	8.74	146.50	10.00	794.35	75.54	4.55	203.66	0.62
46	15.02	147.60	9.60	792.95	67.02	5.44	203.15	1.03
47	8.55	151.29	9.95	792.45	74.13	--	210.39	1.00
48	13.53	140.34	7.63	792.71	52.69	7.19	189.45	1.61
49	17.51	126.66	8.86	796.12	62.81	11.06	172.61	0.80
50	16.96	119.28	9.10	804.41	60.16	10.66	160.09	1.82
51	12.82	124.59	8.49	785.56	55.08	5.29	170.42	1.74
52	14.40	134.92	7.93	802.68	53.22	8.23	179.91	0.97
53	13.39	156.58	7.21	782.13	44.78	9.01	212.16	1.82
MEAN	14.34	127.05	9.12	791.00	63.50	5.99	175.39	1.41
M_1*	12.33	117.62	8.59	786.83	58.07	3.67	161.44	1.08
M_2**	16.35	136.48	9.65	795.21	68.99	8.31	189.35	1.74

Appendix Table 3 e. Raw data for cardiac muscle of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (ml/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
2	m 62.31	54.32	53.09	813.40	353.40	6.08	116.20	11.54
3	64.43	56.13	---	803.90	---	--	---	---
8	77.14	68.24	49.38	822. 6	348.60	61.81	143.27	10.41
9	---	---	49.54	806.82	---	--	---	---
33	71.34	46.74	56.32	808.51	385.90	24.72	1 9.33	13.35
34	63.76	43.15	50.01	811.06	342.70	22.94	90.41	10.68
35	59.38	42.02	48.95	821.05	342.40	15.19	86.33	10.22
39	71.94	53.64	43.38	814.81	295.40	52.68	101.63	8.36
40	52.29	88.67	48.09	819.77	-----	---	---	--
45	26.52	97.77	52.48	---	373.50	---	---	--
46	23.67	104.72	42.15	810.81	287.30	---	198.96	8.05
47	35.28	104.03	39.12	838.70	283.40	20.77	186.26	7.07
48	60.31	88.92	45.06	805.82	331.90	18.15	185.23	9,49
49	57.49	52.16	47.22	817.46	322.60	18.58	104.53	9.55
50	63.38	---	43.89	829.27	306.70	33.27	---	8.41
51	42.41	59.56	43.91	827.27	301.50	---	111.87	8.36
52	56.48	84.28	45.76	824.24	304.30	22.24	161.20	8.81
53	36.07	48.16	37.31	806.06	256.20	---	85.13	6.79
MEAN	54.36	68.28	46.81	816.60	324.80	19/69	129.37	9.48
M_1*	46.15	56.25	44.25	811.57	305.60	8.54	104.72	8.50
M_2**	62.58	80.32	49.36	821.58	343.99	30.84	154. 3	10.46

Appendix Table 3 f. Raw data for liver of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
2	34.44	89.67	55.18	756.90	206.86	---	162.10	49.47
3	35.65	80.38	43.19	---	190.52	---	-----	---
8	33.41	82.12	53.53	752.64	250.02	---	162.97	43.08
9	36.50	89.75	43.27	742.93	221.15	---	170.59	30.57
33	27.42	77.49	55.50	752.84	166.67	---	131.80	57.34
34	30.55	---	57.56	746.88	191.42	---	---	49.54
35	26.86	71.95	49.78	752.78	181.51	---	135.29	46.25
39	33.76	94.79	49.89	762.75	219.64	---	173.58	37.71
40	34.66	83.45	49.32	753.92	---	---	---	---
45	26.25	82.85	46.12	729.16	171.71	---	147.34	43.01
46	12.29	93.86	50.81	750.00	118.34	---	148.23	55.69
47	---	---	41.71	764.71	---	---	---	---
48	34.11	92.58	49.11	762.69	218.17	---	176.85	40.93
49	41.75	82.82	49.07	750.78	230.92	---	179.66	37.55
50	26.74	90.98	50.62	772.49	199.69	---	157.47	43.98
51	32.05	69.14	44.29	756.98	210.12	---	139.57	30.65
52	39.23	89.33	51.96	771.86	264.84	---	175.8	51.83
53	28.62	97.96	51.53	759.96	204.48	---	190.44	56.59
MEAN	32.44	86.23	49.63	758.49	208.40	---	160.72	41.39
M_1*	29.85	81.29	47.96	744.80	187.20		142.68	36.21
M_2**	35.03	91.21	51.34	769.47	229.79		171.89	46.66

*M_1 = 95% Confidence Interval of the Mean
**M_2 = 95% Confidence Interval of the Mean

Appendix Table 3 g. Raw data for gut of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
2	50.31	49.14	34.01	765.80	286.40	---	90.07	6.30
3	56.46	56.55	28.06	790.70	196.60	---	---	4.73
8	55.31	48.35	39.17	802.70	276.50	---	91.40	7.45
9	---	---	43.91	799.90	319.90	---	---	9.16
33	47.78	30.36	45.11	765.71	309.10	---	65.55	9.89
34	46.23	48.18	53.23	758.94	364.80	---	120.07	13.49
35	38.17	53.05	43.00	782.76	300.70	---	68.22	8.93
39	54.72	62.88	46.29	766.65	315.20	---	149.16	11.15
40	29.22	82.95	39.45	789.11	---	---	---	---
45	21.05	69.83	25.76	809.52	190.70	---	112.35	4.33
46	24.17	53.46	42.15	---	287.30	---	---	---
47	21.89	86.07	27.35	813.19	197.70	---	139.16	4.45
48	50.07	---	43.12	790.63	317.60	---	---	9.11
49	56.07	57.06	41.68	799.86	284.80	---	110.04	8.09
50	51.83	83.14	---	814.64	---	---	---	---
51	40.38	43.62	34.55	804.84	237.20	---	75.81	6.09
52	41.58	42.72	35.23	804.41	234.30	---	74.30	6.18
53	34.41	32.04	38.71	804.88	265.08	---	57.89	7.36
MEAN	42.33	56.96	38.92	793.70	270.40	---	92.00	7.78
M_1*	36.00	47.44	35.22	784.20	242.75		70.40	6.35
M_2**	48.66	66.48	42.63	803.06	297.80		113.61	9.21

Appendix Table 3 h. Raw data for brain of rainbow trout acclimated to 18°C (summer series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
2	81.03	53.64	47.71	792.60	317.60	57.99	111.29	10.04
3	80.23	60.22	45.53	811.90	319.10	43.72	---	9.23
8	87.47	77.08	54.59	809.73	385.40	81.52	180.88	12.86
9	---	109.92	57.59	---	419.60	---	---	---
33	75.64	79.44	53.64	812.78	367.60	38.74	177.29	12.05
34	83.21	---	53.52	802.14	366.80	60.79	---	12.28
35	67.35	61.10	48.45	803.65	338.90	33.93	129.98	10.41
39	76.79	81.29	50.72	809.52	345.40	53.15	172.99	10.93
40	60.03	107.28	54.26	812.75	---	---	---	---
45	---	---	---	---	---	---	---	---
46	---	---	---	---	---	---	---	---
47	---	---	---	---	---	---	---	---
48	75.15	107.24	47.26	811.83	348.10	47.49	229.22	10.18
49	74.00	98.56	45.05	814.81	307.80	55.07	193.58	8.89
50	80.46	109.51	46.78	813.19	326.90	64.64	223.77	9.63
51	67.34	94.39	46.91	814.63	322.10	34.77	190.03	9.53
52	71.26	91.05	45.08	805.26	299.80	53.44	179.23	8.92
53	67.19	84.91	44.01	813.83	302.20	42.39	164.11	8.61
MEAN	74.79	86.83	48.88	809.20	340.50	51.36	177.49	10.27
M_1*	70.45	75.79	46.61	805.60	320.60	43.40	154.13	9.44
M_2**	79.14	97.87	51.15	812.80	360.45	59.41	200.84	11.11

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 4 a. Raw data for rainbow trout acclimated to 2°C (fall-winter series)- physical characteristics and plasma electrolyte and water content.

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
56	F	29.0	270.0	124.8	--	137.8	946.0
57	F	30.5	352.5	137.3	0.72	125.3	915.0
58	F	29.0	340.5	150.4	1.10	134.0	934.0
59	M	29.5	275.5	150.4	1.86	140.0	917.0
60	F	28.5	146.0	150.0	0.72	---	924.0
61	F	30.5	310.0	135.2	1.67	128.1	927.0
64	F	29.0	267.5	148.0	1.08	138.9	928.0
65	F	30.5	318.5	148.0	1.75	129.8	930.0
68	F	30.5	316.5	146.4	1.92	138.7	958.0
69	F	29.5	328.5	152.9	3.20	134.0	944.0
74	M	29.0	284.5	152.9	1.54	133.4	950.0
75	F	30.0	321.0	153.7	0.98	126.1	924.0
76	F	31.0	404.5	130.0	0.74	143.5	939.0
77	M	29.5	308.5	151.2	1.18	132.8	942.0
90	-	28.5	288.5	146.7	0.62	133.8	932.0
91	-	29.0	313.0	148.3	0.77	131.7	930.0
92	-	26.5	226.5	144.6	1.15	127.8	940.0
93	-	29.5	309.0	151.0	1.15	141.0	944.0
94	F	30.0	327.5	162.1	2.26	134.0	911.0
95	F	28.0	257.0	163.1	1.80	139.8	938.0
96	F	30.0	338.5	164.6	2.79	130.9	926.0
97	M	29.0	301.5	154.3	1.79	136.3	950.0
116	F	28.0	250.5	134.2	2.00	125.9	940.0
117	F	28.5	311.0	146.4	1.85	136.0	936.0
119	M	28.0	254.5	143.1	1.55	133.6	968.0
120	M	27.5	249.0	155.5	0.95	137.3	946.0
121	F	26.0	196.0	129.8	0.77	120.7	966.0
MEAN		29.1	287.1	147.24	1.45	133.49	937.0
H_1*		28.6	267.3	143.24	1.18	131.23	931.3
H_2**		29.5	307.9	151.22	1.72	135.74	942.7

Appendix Table 4 b. Raw data for post-opercular muscle of rainbow trout acclimated to 2°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	H_2O^{ecs} Cl/K (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
56	11.97	---	6.86	777.24	---	---	---	--
57	11.04	169.11	6.22	772.19	48.44	6.06	233.61	0.21
58	14.63	151.94	6.71	782.88	47.09	10.27	206.15	0.54
59	12.03	155.13	6.14	766.89	38.14	8.69	212.78	1.09
60	8.77	---	5.87	---	---	--	---	--
61	9.84	122.14	6.48	775.81	43.36	5.55	166.66	1.15
64	12.58	130.20	6.63	776.88	44.49	8.19	177.49	0.62
65	11.69	156.28	5.61	773.99	38.14	3.21	212.29	0.90
68	--	149.04	8.19	767.67	53.46	--	208.45	1.08
69	16.74	148.25	7.64	762.08	46.63	13.43	207.00	1.95
74	14.75	136.01	7.47	769.43	51.12	9.65	189.24	0.91
75	---	130.29	7.05	775.33	53.06	--	180.32	0.49
76	---	---	6.76	771.38	---	--	---	--
77	12.09	152.01	6.63	774.10	46.74	6.91	208.91	0.58
90	11.92	142.76	7.10	769.64	51.94	5.99	198.87	0.21
91	10.93	148.17	7.55	767.58	51.48	4.60	206.86	1.08
92	8.75	143.40	4.98	775.14	35.39	4.91	193.79	0.64
93	11.75	142.56	7.09	781.93	46.84	6.68	193.86	0.66
94	12.44	146.96	6.76	783.15	42.69	7.45	198.34	1.40
95	11.61	149.67	6.53	775.77	40.92	6.99	203.57	1.01
96	8.76	142.14	6.90	778.23	35.43	3.94	191.22	1.84
97	14.05	146.54	7.14	774.76	46.75	9.39	201.17	1.05
116	9.83	143.86	5.26	775.24	40.54	5.17	196.23	1.17
117	14.59	146.26	8.31	775.46	55.65	8.98	203.61	1.04
119	12.99	149.17	6.49	774.42	43.84	9.19	204.09	0.87
120	12.51	140.62	6.18	778.04	42.19	8.09	191.06	0.53
121	7.41	148.43	5.14	786.17	40.75	2.86	199.08	0.30
MEAN	11.81	145.46	6.63	774.50	44.80	7.41	199.27	0.97
H_1*	10.86	141.44	6.30	772.29	42.40	6.33	193.62	0.78
H_2**	12.75	149.47	6.96	776.71	47.20	8.50	204.92	1.16

*H_1 - 95% Confidence interval lower limit
**H_2 - 95% Confidence interval upper limit

Appendix Table 4 c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 2°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (ml/kg)	H_2O (ml/kg)	H_2O^{ecs} Cl/K (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
56	--	168.97	5.41	767.78	---	--	--	--
57	7.67	145.36	5.03	770.36	38.17	3.29	198.52	0.32
58	11.85	140.98	5.99	780.07	41.74	7.62	190.75	0.63
59	10.21	153.58	5.68	758.64	34.79	6.88	212.08	1.12
60	8.06	151.51	4.98	778.39	---	--	---	--
61	9.90	153.49	6.00	776.58	40.76	6.19	181.32	1.06
64	9.81	133.54	5.21	770.83	34.08	6.46	181.21	0.65
65	10.59	159.99	4.92	773.15	32.51	7.80	215.94	0.95
68	16.79	155.30	--	768.52	---	--	---	--
69	14.39	148.79	6.86	---	---	--	---	--
74	11.33	139.76	6.14	772.69	40.85	6.95	190.89	0.94
75	10.72	138.50	6.11	767.94	45.68	5.12	191.69	0.48
76	10.50	150.69	5.88	762.76	39.21	7.47	208.22	0.35
77	9.48	162.95	5.44	770.78	---	--	---	--
90	9.55	157.77	5.70	772.06	41.38	4.76	215.99	0.22
91	8.68	151.29	5.51	767.14	39.97	3.79	208.01	0.54
92	7.54	149.98	4.30	769.17	30.13	4.31	202.89	0.63
93	9.58	145.38	5.59	778.85	35.95	5.32	195.64	0.70
94	8.99	147.00	5.17	779.41	30.34	5.44	196.15	1.47
95	10.13	152.98	5.77	770,80	35.49	5.91	207.96	1.09
96	5.87	---	5.55	777.52	---	--	---	--
97	12.47	150.45	6.20	767.11	39.88	8.69	206.78	1.05
116	8.88	146.55	5.26	769.31	34.96	5.71	199.47	1.17
117	11.33	155.45	6.06	769.96	38.86	7.72	212.53	1.06
119	10.91	148.58	5.81	770.79	38.58	7.36	202.84	0.89
120	10.20	144.95	5.96	773.71	38.66	5.69	197.15	0.52
121	9.66	147.86	5.95	785.91	47.64	4.71	200.23	0.27
MEAN	10.19	148.52	5.62	771.93	38.06	6.06	200.77	0.76
M_1*	9.32	144.89	5.41	769.58	36.03	5.40	196.17	0.60
M_2**	11.06	152.15	5.83	774.28	40.08	6.61	205.37	0.92

Appendix Table 4 d. Raw data for caudal muscle of rainbow trout acclimated to 2°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	H_2O^{ecs} Cl/K (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
56	9.90	176.37	7.12	776.22	---	--	----	--
57	11.55	157.42	6.44	768.71	50.10	6.50	219.11	0.23
58	13.88	154.00	7.11	780.10	50.27	8.68	210.93	0.51
59	12.74	173.33	6.77	760.29	45.65	8.62	241.75	0.92
60	9.19	152.02	5.95	781.02	---	---	---	--
61	11.47	130.08	7.51	774.92	52.91	6.12	179.82	1 01
64	11.69	136.95	6.67	774.72	45.03	6.89	187.62	0.57
65	13.25	166.38	6.56	775.24	45.86	8.36	228.00	0.83
68	---	148.81	7.96	764.59	51.79	--	203.63	1.09
69	14.90	152.64	8.06	758.91	50.39	10.15	215.21	1.85
74	10.64	128.81	7.17	774.15	48.33	4.48	177.38	0.99
76	10.68	143.73	6.63	772.88	50.04	4.14	198.77	0.33
77	11.75	147.53	6.89	770.01	46.36	7.91	203.82	0.33
90	11.68	146.60	6.54	772.88	45.89	6.52	201.58	0.61
91	10.75	175.18	7.28	777.52	53.72	3.96	241.98	0.13
92	9.25	149.55	6.53	765.93	42.93	2.98	208.24	0.30
93	7.98	146.59	6.72	773.88	49.61	1.11	200.93	0.56
94	11.52	140.83	7.06	775.99	46.64	6.14	193.02	0.65
95	11.19	151.45	6.81	781.11	43.40	5.63	205.16	1.35
96	12.05	153.27	7.09	774.67	45.28	6.39	210.02	1.04
97	10.97	169.52	6.29	775.33	39.79	6.03	230.95	1.47
116	14.16	147.82	7.68	777.21	50.89	8.68	202.39	0.48
117	10.54	153.39	6.59	773.42	46.76	5.96	210.82	1.05
119	12.81	150.57	7.60	770.00	50.45	7.59	209.13	1.03
120	10.51	152.69	6.42	778.96	43.41	5.85	209.11	0.85
121	11.85	139.38	7.62	775.79	53.25	4.94	192.83	0.47
MEAN	11.33	150.84	6.95	772.50	48.50	6.27	207.88	0.77
M_1*	10.64	145.28	6.72	768.60	46.68	5.36	201.21	0.59
M_2**	12.03	155.89	7.18	776.36	50.30	7.18	214.54	0.95

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 4 e. Raw data for cardiac muscle of rainbow trout acclimated to 2°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
56	49.89	x 32.00	31.44	813.98	205.34	39.93	---	5.17
57	41.91	45.42	28.05	799.76	201.48	23.23	75.76	4.69
58	50.43	40.60	32.77	808.53	226.81	36.65	27.06	5.81
59	43.69	46.74	30.53	782.88	196.26	23.91	78.25	5.15
60	44.59	55.18	---	796.04	---	---	---	---
61	36.42	27.64	29.02	787.69	203.89	15.59	45.97	4.89
64	44.30	45.35	35.52	798.46	230.15	17.84	79.39	6.25
65	47.39	47.29	33.62	801.52	233.11	22.68	83.52	5.92
68	51.58	59.85	34.91	807.38	226.52	31.71	102.29	6.01
69	52.51	53.04	---	805.76	---	---	---	---
74	40.21	47.43	31.16	801.50	210.22	13.64	79.66	5.27
75	42.55	50.18	33.59	802.07	239.74	10.14	88.82	5.97
76	43.42	50.46	32.12	805.64	201.45	28.52	85.27	5.32
77	47.47	40.75	30.70	798.34	221.61	24.21	70.17	5.67
90	35.52	21.86	30.23	809.03	230.34	6.09	35.89	4.99
91	40.17	28.34	32.23	810.05	230.25	12.74	47.76	5.46
92	36.58	40.30	32.47	801.92	229.56	5.96	69.95	5.76
93	45.43	23.07	34.33	803.99	223.32	20.22	38.89	5.73
94	41.43	29.30	26.40	803.73	177.31	20.25	46.13	4.22
95	47.03	40.39	36.82	809.59	237.04	14.62	69.79	6.43
96	32.90	23.58	35.82	811.28	232.53	---	39.62	5.84
97	47.47	27.49	30.23	820.02	299.61	26.87	43.73	4.87
116	29.18	27.25	27.78	802.24	198.59	4.19	44.45	4.60
117	---	32.15	37.72	797.64	249.62	---	57.82	6.88
119	39.18	34.12	29.14	788.89	196.30	18.71	57.06	4.92
120	46.57	38.04	33.64	810.11	220.51	22.52	64.16	5.71
121	---	59.66	27.20	801.36	202.82	---	99.43	4.54
MEAN	43.19	39.19	31.96	803.75	215.45	20.04	65.42	5.44
M_1*	40.56	34.63	30.72	800.50	208.25	15.84	57.18	5.18
M_2**	45.81	43.74	33.19	806.99	222.66	24.24	73.67	5.71

Appendix Table 4 f. Raw data for liver of rainbow trout acclimated to 2°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
56	30.37	---	38.39	758.53	258.33	---	---	---
57	26.94	134.68	32.45	732.78	208.29	---	256.49	12.11
58	34.54	122.29	38.82	739.38	243.79	---	246.22	12.41
59	36.06	127.73	38.47	735.17	254.52	---	264.26	5.90
60	28.89	---	38.50	752.62	235.91	---	---	---
61	22.49	96.02	37.09	737.76	179.24	---	171.38	25.29
64	27.46	95.72	41.69	742.41	196.96	---	175.13	26.28
65	29.54	108.22	38.14	730.61	211.88	---	207.92	20.51
68	35.62	129.82	---	755.63	258.29	---	---	---
69	---	94.95	48.94	733.68	---	---	---	---
74	29.43	124.50	35.67	720.89	204.33	---	235.84	15.98
76	27.83	123.99	35.43	734.95	192.21	---	228.11	20.62
77	29.69	115.14	40.32	742.94	243.45	---	229.69	11.05
90	29.99	115.77	35.67	737.29	210.56	---	219.32	14.63
91	31.34	113.19	36.86	744.97	226.78	---	218.16	12.58
92	28.55	134.18	38.87	742.63	204.37	---	248.89	22.21
93	27.54	106.59	35.74	744.46	202.18	---	196.13	18.46
94	26.55	112.22	43.13	735.99	186.65	---	203.89	30.60
95	28.14	118.95	32.21	758.82	194.28	---	206.31	13.08
96	27.82	104.81	39.89	737.69	181.07	---	187.91	26.22
97	29.79	127.91	35.86	744.04	192.13	---	230.79	19.41
116	31.68	113.04	39.47	745.63	217.95	---	212.48	18.51
117	30.19	111.72	37.03	744.76	228.81	---	200.85	13.74
119	29.95	119.90	38.18	746.60	217.17	---	224.86	16.27
120	26.79	117.98	34.86	733.14	195.03	---	218.69	16.36
121	33.53	104.52	39.80	758.69	228.90	---	196.88	15.81
MEAN	29.20	115.93	38.06	742.45	215.54	---	218.26	17.64
M_1*	27.96	111.32	36.65	738.92	203.38		207.44	15.01
M_2**	30.66	120.64	39.47	745.97	227.71		229.08	20.26

*M_1 - 95% confidence interval lower limit
**M_2 - 95% confidence interval upper limit

Appendix Table 4 g. Raw data for spleen of rainbow trout acclimated to 7°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
56	20.96	70.26	32.27	737.35	178.29	---	---	24.04
57	21.39	108.90	37.58	739.78	165.38	---	190.07	29.63
58	21.19	110.57	31.76	709.80	149.56	---	207.78	20.92
59	24.57	106.16	36.88	751.44	177.42	---	189.67	22.58
60	18.02	85.65	41.11	734.90	141.15	---	145.54	---
61	18.03	111.63	43.48	743.51	165.69	---	186.00	41.87
64	16.09	81.95	34.49	716.85	115.41	---	136.06	30.69
65	21.29	114.74	37.76	721.55	153.22	---	201.24	31.53
68	21.96	115.24	---	766.41	159.23	---	---	---
69	26.91	98.59	---	---	186.83	---	---	---
74	17.44	92.56	34.47	741.61	121.08	---	148.86	29.52
76	20.17	113.09	30.30	715.23	139.31	---	196.13	22.11
77	20.08	108.37	34.45	724.81	163.97	---	193.01	19.44
90	25.68	125.36	35.28	729.86	180.29	---	227.71	20.63
91	13.53	98.52	35.13	727.32	97.91	---	156.43	35.00
92	17.99	107.88	33.27	714.97	128.78	---	183.87	27.82
93	16.88	75.00	34.59	750.93	123.92	---	119.39	30.01
94	19.32	110.42	29.20	709.68	135.87	---	192.14	17.87
95	21.99	105.44	34.50	743.52	144.01	---	174.98	25.36
96	21.82	104.05	34.49	719.72	142.02	---	179.65	25.33
97	18.07	87.93	34.13	754.94	116.54	---	139.23	29.57
116	21.23	100.38	36.10	746.46	146.06	---	166.75	26.97
117	17.86	90.44	31.67	719.19	141.28	---	156.01	24.02
119	17.64	74.49	34.90	728.62	127.91	---	123.61	29.14
120	17.72	70.89	34.39	753.27	131.45	---	117.45	27.62
121	16.54	73.74	34.51	728.53	112.91	---	119.61	30.88
MEAN	19.53	97.21	34.92	732.00	142.20	---	165.59	27.02
H_1*	18.24	90.85	33.61	726.01	135.21		151.95	24.77
H_2**	20.83	103.57	36.22	738.06	151.24		179.23	29.27

Appendix Table 4 h. Raw data for gut of rainbow trout acclimated to 2° (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
56	58.48	36.02	49.49	761.49	323.23	---	----	11.29
57	41.13	34.28	---	751.50	----	---	----	---
58	35.85	60.91	47.68	763.60	320.24	---	136.59	10.75
59	56.13	70.06	58.52	760.44	376.20	---	180.51	15.23
60	62.29	63.62	52.39	795.27	---	---	----	---
61	48.78	57.35	48.59	776.28	342.08	---	130.77	11.21
64	47.55	48.21	47.55	798.35	308.09	---	97.21	9.70
65	52.75	36.59	49.17	745.67	340.93	---	88.95	12.15
68	61.37	---	58.09	776.14	376.93	---	---	14.55
69	64.10	61.85	59.68	766.90	400.84	---	165.46	16.30
74	48.72	59.89	41.48	749.26	279.85	---	126.67	8.84
76	58.25	41.13	54.08	761.56	385.98	---	108.50	14.39
77	57.59	56.65	51.79	769.37	324.81	---	126.89	11.65
90	55.85	57.99	53.24	756.20	360.81	---	135.32	12.52
91	60.12	38.05	63.65	780.37	428.14	---	107.22	18.07
92	57.36	59.39	61.77	793.36	402.12	---	158.97	16.62
93	54.04	24.19	55.51	779.04	393.45	---	61.41	14.36
94	63.79	25.76	63.35	788.21	404.38	---	69.89	16.51
95	48.49	58.02	55.01	771.63	368.47	---	142.19	13.68
96	58.93	30.52	65.59	783.69	422.25	---	82.84	18.15
97	54.39	38.55	55.27	796.53	379.94	---	89.99	13.26
116	62.90	46.02	62.23	760.45	410.91	---	129.55	17.80
117	53.68	26.49	58.02	767.81	414.76	---	72.69	16.43
119	52.90	44.45	52.55	750.97	347.76	---	108.64	13.03
120	63.25	38.36	61.05	754.09	411.26	---	101.99	17.60
121	58.67	35.31	61.06	763.38	400.75	---	96.19	16.81
MEAN	54.91	45.45	55.38	771.36	378.75	---	116.09	14.14
H_1*	52.10	39.97	52.82	765.33	356.13		99.33	12.01
	57.73	50.92	57.93	777.39	389.37		132.86	15.26

*H_1 - 95% Confidence Interval lower limit
**H_2 - 95% Confidence Interval upper limit

Appendix Table 4 i. Raw data for brain of rainbow trout acclimated to 7°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (ml/kg)	Cl^- (ml/kg)	H_2O (ml/kg)	$H_2O^{sce}Cl$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell}	Cl^-_{cell}
56	65.44	46.78	39.48	798.49	257.85	61.52	---	7.30
57	65.25	---	51.47	809.56	369.69	33.02	---	11.73
58	66.85	46.11	39.34	79 .56	264.22	51.02	86.23	7.40
59	77.79	58.55	46.46	818.27	298.67	63.76	73.12	8.94
60	53.03	26.59	27.79	825.11	---	---	---	--
61	51.39	32.07	35.41	801.15	234.73	35.53	55.92	5.89
64	59.05	31.19	38.23	806.74	247.71	40.05	55.35	6.84
65	57.77	47.82	36.02	792.50	249.75	38.34	87.31	6.64
68	79.65	---	51.64	806.50	335.08	64.89	---	10.95
69	82.52	30.37	52.49	824.16	352.54	60.68	62.00	11.13
74	61.22	35.97	39.55	805.92	266.83	37.88	65.96	7.34
75	64.68	31.14	40.65	798.59	290.13	39.51	60.68	7.99
76	67.79	33.32	39.11	804.16	245.29	64.24	59.29	6.99
77	56.87	40.90	38.07	800.99	258.00	32.89	74.75	7.01
90	65.54	33.48	51.03	818.48	343.25	31.95	70.00	10.74
91	59.71	21.97	43.64	805.42	298.22	30.53	42.86	8.60
92	58.93	24.94	45.36	795.09	320.69	26.47	51.79	9.56
93	49.52	29.28	41.89	812.45	267.38	16.41	53.15	7.71
94	84.89	33.51	---	---	---	---	---	--
95	43.54	27.11	34.67	815.64	223.19	12.05	45.08	5.85
96	59.51	26.16	39.63	818.39	272.47	26.86	46.53	7.26
97	73.56	21.06	52.24	815.70	344.94	43.19	43.42	11.09
116	44.63	30.23	41.59	802.27	297.31	9.37	58.69	8.24
117	76.80	37.27	46.03	809.09	304.61	65.84	72.76	9.12
119	76.96	44.25	47.98	807.95	323.22	63.35	90.25	10.11
120	75.97	26.25	47.19	809.60	309.33	55.71	51.88	9.43
121	57.99	28.92	41.01	805.56	305.79	36.61	57.43	8.21
MEAN	64.32	33.01	42.54	807.80	291.20	41.57	62.02	8.48
M_1*	59.85	29.88	39.96	804.24	274.77	34.52	55.79	7.77
M_2**	68.79	36.14	45.11	811.32	307.70	48.61	68.26	9.20

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 5 a. Raw data for rainbow trout acclimated to 10°C (fall-winter series) - physical characteristics and plasma electrolytes and water content.

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
66	F	29.0	161.0	161.0	1.12	130.9	924.0
67	M	31.5	420.0	152.9	0.62	127.8	924.0
70	M	30.5	262.5	146.4	3.03	138.1	936.0
71	M	31.0	388.3	150.4	0.53	137.4	938.0
72	F	29.5	324.0	150.5	1.06	130.2	920.0
80	M	29.0	356.0	150.6	1.00	133.1	924.0
81	F	30.0	335.0	153.9	1.21	132.6	962.0
84	M	30.0	370.0	156.0	2.46	128.8	940.0
85	F	29.5	303.5	159.3	0.72	132.7	947.0
88	F	31.0	392.0	147.8	1.21	130.7	946.0
89	-	30.0	362.0	153.7	0.84	130.4	924.0
102	M	28.5	332.0	149.7	3.03	131.3	947.0
103	F	29.0	316.0	153.7	1.97	136.8	952.0
104	F	28.5	267.0	154.1	1.37	134.0	936.0
105	M	30.0	358.0	158.5	1.39	130.6	936.0
110	M	28.0	238.0	163.9	1.08	136.4	936.0
111	-	31.5	355.0	151.0	2.08	135.2	938.0
112	F	27.5	253.0	153.0	1.85	138.2	939.0
113	F	27.5	232.0	154.8	1.22	138.0	946.0
114	M	32.0	363.5	152.1	3.21	133.5	928.0
115	M	27.5	237.5	145.7	1.85	128.4	952.0
MEAN		29.6	323.0	153.57	1.56	133.10	937.9
M_1*		28.9	297.6	151.46	1.20	131.56	932.8
M_2**		30.2	348.4	155.67	1.93	134.64	942.9

Appendix Table 5 b. Raw data for post-opercular muscle of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg).	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl\ K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
66	14.86	158.28	7.70	771.74	56.36	8.09	221.16	0.45
67	12.03	149.43	8.54	770.54	66.21	9.81	212.10	0.11
70	16.17	152.65	7.01	769.28	40.78	14.00	209.37	1.89
71	13.24	154.77	7.56	777.24	54.42	6.99	214.03	0.11
72	11.81	154.69	7.46	764.73	54.98	4.98	217.87	0.43
80	15.39	143.23	8.54	763.23	62.03	8.09	204.18	0.41
81	13.01	144.78	7.38	761.46	52.51	6.95	204.13	0.59
84	18.26	136.69	7.34	762.44	48.59	14.96	191.32	1.52
85	14.19	141.15	7.01	---	---	---	---	---
88	12.01	152.08	6.94	766.91	50.01	6.46	212.05	0.56
89	16.53	144.22	9.10	761.35	68.46	8.67	208.06	0.25
102	16.44	147.95	8.90	784.27	57.91	10.69	203.45	1.78
103	13.27	144.34	8.09	778.98	52.89	7.08	198.65	1.18
104	10.61	155.06	6.77	772.54	46.78	4.69	213.57	0.69
105	16.40	137.44	8.81	778.04	63.57	8.85	192.24	0.71
110	9.85	139.38	8.15	772.79	56.95	---	194.65	0.53
111	10.99	151.11	7.21	767.77	46.99	5.40	209.51	1.19
112	9.98	136.06	7.44	781.99	47.32	3.73	185.08	1.22
113	15.98	142.43	6.99	774.65	47.03	9.21	195.67	0.69
114	14.93	138.25	7.51	769.61	44.63	11.23	190.49	2.14
115	11.37	145.42	7.04	779.09	48.92	5.81	199.04	1.04
MEAN	13.82	146.16	7.69	771.43	53.37	7.82	203.83	0.87
M_1*	12.69	143.07	7.36	768.17	49.86	6.58	199.11	0.59
M_2**	14.96	149.25	8.02	774.68	56.88	9.53	208.56	1.15

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 5 c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	H_2O^{ecs} Cl/K (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
66	12.49	155.66	6.44	768.28	46.38	6.96	215.55	0.51
67	---	154.96	7.64	766.95	59.06	--	218.85	0.13
70	11.02	150.41	5.27	769.28	31.12	8.76	203.64	1.99
71	11.39	138.30	7.08	775.70	50.60	5.21	190.69	0.18
72	---	148.22	6.61	762.97	48.09	--	207.26	0.49
80	12.27	149.79	6.94	758.96	49.81	6.30	211.15	0.44
81	11.09	148.11	6.62	759.46	46.69	5.48	207.22	0.60
84	13.05	159.02	6.06	760.82	39.62	9.52	220.36	1.33
85	11.17	148.09	5.78	770.19	41.85	6.18	205.28	0.31
88	10.22	157.51	5.11	768.14	35.65	6.83	214.97	--
89	14.58	149.01	7.23	767.32	53.71	8.86	208.75	0.32
102	13.61	145.82	7.04	778.16	42.96	9.76	198.16	1.90
103	11.25	156.64	6.87	775.40	44.27	6.08	214.12	1.11
104	10.17	163.46	5.51	774.04	37.26	6.01	221.79	0.70
105	13.93	148.65	7.51	777.94	53.65	7.49	205.13	0.69
110	9.86	156.49	--	763.60	---	---	---	--
111	11.62	163.61	6.31	767.78	40.71	7.57	225.79	1.11
112	11.29	158.14	6.75	773.75	43.37	6.38	216.41	1.04
113	10.02	152.40	6.23	772.03	41.68	4.89	208.59	0.67
114	11.86	146.95	6.36	765.88	36.43	8.66	201.29	2.05
115	12.61	154.75	6.17	771.40	42.45	8.81	212.19	0.99
MEAN	11.76	152.65	6.50	768.80	44.27	7.21	210.29	0.87
M_1*	11.12	149.84	6.19	766.10	41.04	6.45	206.26	0.59
M_2**	12.41	155.46	6.81	771.42	47.49	7.97	214.31	1.1

Appendix Table 5 d. Raw data for caudal muscle of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	H_2O^{ecs} Cl/K (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
66	13.79	---	7.81	772.84	---	--	---	--
67	16.78	160.97	7.77	771.38	60.16	10.66	226.28	0.12
70	13.91	148.05	7.13	772.64	41.23	10.77	202.25	1.96
71	14.40	157.54	7.59	776.16	54.68	8.56	218.31	0.11
72	14.11	151.61	7.01	760.98	51.37	8.99	213.58	0.45
80	12.86	149.43	7.28	761.53	52.41	6.56	209.71	0.43
81	11.46	148.61	7.29	758.74	51.96	4.90	210.78	0.57
84	13.36	134.27	6.58	768.73	42.02	9.37	184.62	1.61
85	11.39	158.82	6.45	799.42	47.03	5.19	211.04	0.28
88	11.92	156.25	7.23	767.77	52.42	5.83	218.34	0.53
89	14.66	137.86	7.57	772.26	56.10	8.43	192.43	0.34
102	15.69	151.15	--	744.26	---	--	---	--
103	14.19	168.71	7.99	777.26	53.29	8.29	232.89	0.97
104	11.55	160.24	7.19	775.04	50.15	5.27	220.96	0.68
105	13.21	138.70	8.09	782.04	57.83	5.58	191.41	0.74
110	10.92	153.60	6.74	773.29	46.64	5.15	211.59	0.52
111	12.98	156.77	7.25	773.36	47.47	8.01	219.83	1.15
112	16.38	150.97	--	781.45	---	--	---	--
113	10.96	158.67	7.13	779.78	48.35	4.75	208.65	0.63
114	13.97	146.21	7.55	770.04	45.66	9.69	201.66	2.08
115	13.63	162.47	7.04	774.81	49.77	8.79	223.96	0.89
MEAN	13.43	152.21	7.29	770.66	50.47	7.49	210.79	0.78
M_1*	12.67	148.16	7.08	763.60	47.95	6.46	204.51	0.49
M_2**	14.19	156.26	7.51	777.70	52.99	8.52	217.08	1.07

*M_1 -95% Confidence Interval lower limit
**M_2 -95% Confidence Interval upper limit

Appendix Table 5 e. Raw data for cardiac muscle of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
66	--	49.30	34.20	804.16	235.14	--	82.27	5.74
67	50.30	34.23	35.18	819.80	247.75	21.71	59.57	6.15
70	43.87	34.78	31.72	814.29	2 6.72	22.39	56.21	5.22
71	49.84	---	32.68	808.34	214.06	26.69	---	5.49
72	48.85	38.23	37.68	803.14	260.46	17.62	69.29	6.88
80	43.58	49.13	33.49	804.94	226.45	14.03	84.54	5.79
81	47.54	47.79	33.65	807.06	228.39	21.41	82.11	5.82
84	42.28	60.12	28.94	812.68	202.22	17.58	97.67	4.74
85	50.83	36.93	36.21	---	245.58	---	---	--
88	46.38	35.70	29.90	798.19	205.89	26.93	59.86	5.05
89	45.47	60.98	28.79	807.84	198.70	24.51	99.83	4.73
102	46.22	67.26	28.64	808.86	196.31	27.48	108.83	4.68
103	42.33	34.17	33.41	827.07	219.80	14.07	55.55	5.50
104	46.81	39.43	35.27	814.63	236.89	18.48	67.87	6.10
105	48.32	56.79	28.23	808.95	194.54	28.46	91.99	4.59
110	41.48	35.62	34.33	804.08	226.52	7.54	61.25	5.94
111	52.87	56,92	32.68	810.84	217.59	33.75	95.18	5.49
112	59.86	40.37	36.82	850.95	239.78	37.92	63.33	6.03
113	34.31	69.70	32.48	766.92	211.83	2.74	125.09	5.85
114	---	48.59	---	814.17	---	---	---	--
115	47.07	37.21	32.26	795.29	226.12	24.82	64.64	5.67
MEAN	46.75	46.66	32.83	807.10	222.00	20.12	79.28	5.55
M_1*	44.21	41.18	31.52	801.60	213.35	15.36	69.18	5.26
M_2**	49.28	52.14	34.13	812.60	230.72	24.88	89.39	5.84

Appendix Table 5 f. Raw data for liver of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
66	34.90	125.79	42.83	734.99	230.11	---	248.64	25.71
67	38.61	133.67	42.26	726.59	268.06	---	290.90	28.33
70	35.20	108.83	42.98	725.39	255.24	---	229.83	16.42
71	32.86	118.54	41.70	739.48	231.94	---	233.32	19.37
72	40.95	117.68	50.12	759.31	288.85	---	249.49	26.59
80	34.50	116.19	---	721.09	233.87	---	---	---
81	32.83	115.66	43.69	733.87	226.45	---	227.39	26.93
84	33.15	122.06	---	730.79	225.58	---	---	---
85	38.38	---	41.67	725.60	255.76	---	---	16.45
88	26.23	120.02	40.05	718.08	188.39	---	226.15	29.15
89	30.02	114.53	45.28	726.04	207.34	---	220.47	35.17
102	29.29	126.53	44.03	726.88	207.70	---	242.49	32.38
103	37.26	115.33	50.22	755.13	257.35	---	230.67	30.16
104	35.53	119.16	45.92	750.39	244.76	---	235.01	25.95
105	40.91	125.93	45.09	733.62	273.99	---	273.03	20.24
110	---	110.46	40.05	756.81	---	---	---	---
111	37.19	122.41	46.28	763.12	261.45	---	242.92	21.79
112	42.27	110.72	49.28	764.01	293.28	---	234.06	18.58
113	35.10	135.51	43.90	760.35	240.70	---	260.21	20.56
114	35.76	127.29	41.65	745.63	249.58	---	254.99	16.79
115	32.98	126.64	45.07	746.19	247.58	---	253.07	26.65
MEAN	35.25	120.65	44.58	739.75	244.40	---	247.21	24.29
M_1*	33.37	117.19	43.10	732.63	231.90		237.22	21.45
M_2**	37.12	124.10	46.07	746.86	256.90		257.21	27.13

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 5 g. Raw data for spleen of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
66	18.30	95.24	43.34	751.42	120.66	---	150.77	43.67
67	23.64	102.73	44.83	772.16	164.13	---	168.79	39.28
70	18.47	107.59	40.45	731.82	133.93	---	178.94	36.72
71	22.59	102.37	41.97	771.71	159. 5	---	167.06	32.77
72	---	121.18	---	---	---	---	---	---
80	24.11	112.79	44.61	763.59	163.44	---	187.66	38.08
81	22.53	121.85	41.58	747.86	155.41	---	205.36	35.39
84	20.11	76.84	41.09	---	136.85	---	---	---
85	20.78	115.42	40.23	760.30	138.48	---	185.46	35.14
88	22.86	115.47	42.04	755.26	164.19	---	195.02	34.82
89	20.43	118.33	42.79	765.68	141.10	---	189.27	39.05
102	21.23	94.93	41.48	746.83	150.55	---	158.44	36.42
103	21.69	110.19	37.59	728.05	149.81	---	190.05	29.57
104	17.87	87.04	39.92	733.86	123.10	---	142.25	38.35
105	18.42	75.69	44.75	760.79	123.37	---	118.48	44.93
110	14.38	73.68	43.16	755.79	93.14	---	111.04	45.96
111	21.46	96.39	41.98	746.67	150.86	---	161.25	36.23
112	16.03	100.67	40.85	743.24	111.22	---	158.96	40.31
113	14.07	74.76	40.71	747.27	96.49	---	114.69	42.09
114	21.54	107.34	40.70	761.25	150.34	---	174.92	33.77
115	22.25	93.07	41.10	757.68	162.11	---	155.77	34.06
MEAN	20.14	100.15	41.76	752.70	139.40	---	163.90	37.72
M_1*	18.78	93.05	40.92	746.60	129.16		150.72	35.65
M_2**	21.49	107.25	42.59	758.80	149.70		177.09	39.78

Appendix Table 5 h. Raw data for gut of rainbow trout acclimated to 10°C (fall-winter sereis).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
66	44.02	34.07	44.33	---	308.23	---	---	---
67	54.62	61.94	52.30	782.41	368.31	---	149.03	12.63
70	43.90	59.43	47.61	802.36	310.27	---	118.86	9.68
71	42.32	79.49	60.55	805.39	396.61	---	193.97	14.82
72	56.60	41.08	55.15	776.90	381.22	---	102.80	13.94
80	62.25	72.84	65.41	774.91	442.29	---	217.62	19.67
81	53.83	83.26	55.99	783.45	380.02	---	205.24	13.88
84	56.28	34.48	52.51	732.69	366.92	---	91.79	14.35
85	49.01	33.44	51.76	735.50	351.05	---	86.32	13.46
88	61.60	48.25	61.27	745.99	421.90	---	148.22	19.02
89	68.08	47.98	66.84	778.31	461.32	---	150.14	21.09
102	68.17	45.39	79.78	760.07	546.85	---	205.11	37.42
103	68.21	63.28	72.24	759.26	475.26	---	219.52	25.44
104	58.34	48.55	57.75	778.13	387.87	---	123.04	14.79
105	67.72	39.39	70.38	755.09	485.08	---	143.39	26.03
110	62.99	54.54	68.42	773.79	451.45	---	167.69	21.23
111	74.31	56.10	71.43	757.54	475.49	---	195.39	25.33
112	72.52	36.80	75.53	781.01	491.87	---	124.15	25.92
113	68.31	36.48	66.87	757.47	449.15	---	116.54	22.34
114	50.06	54.66	54.90	754.09	370.11	---	139.26	14.29
115	46.77	36.80	53.14	758.29	372.48	---	92.64	13.63
MEAN	59.21	50.87	61.27	767.70	413.99	---	149.54	18.95
M_1*	54.47	44.05	56.83	758.60	385.56		129.05	15.81
M_2**	63.95	57.69	65.70	776.84	442.62		170.02	22.09

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 5 i. Raw data for brain of rainbow trout acclimated to 10°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ECS}_{Cl}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
66	64.73	---	44.04	816.99	302.79	31.08	---	8.57
67	80.56	45.01	50.91	806.07	358.52	57.51	100.08	11.37
70	83.88	38.04	56.41	---	367.62	---	---	---
71	87.57	30.55	47.09	808.08	308.45	82.42	60.82	9.43
72	---	35.23	64.31	820.75	444.54	---	92.39	17.09
80	70.34	3 .01	46.99	800.99	317.74	42.59	67.63	9.72
81	69.54	33.81	49.49	811.56	335.00	37.52	70.23	10.41
84	65.69	30.22	41.00	811.18	286.49	40.02	56.25	7.81
85	61.24	29.19	44.89	792.29	304.45	26.12	59.39	9.21
88	65.95	42.67	47.21	818.77	325.09	36.26	85.64	9.56
89	68.96	45.69	55.93	822.29	386.02	22.07	103.99	12.82
102	77.46	42.95	56.66	826.56	388.38	44.09	95.53	12.93
103	95.49	28.36	59.26	825.49	389.87	81.64	63.34	13.61
104	73.53	33.45	49.68	824.28	353.67	45.07	67.24	9.29
105	64.40	37.98	45.85	808.43	315.96	29.08	76.23	9.31
110	64.44	37.28	47.39	808.26	312.69	26.62	74.39	9.56
111	73.78	30.85	47.42	814.60	315.66	52.34	60.52	9.51
112	87.98	39.13	58.34	836.11	379.93	65.44	84.24	12.76
113	75.87	35.71	52.53	792.87	342.59	50.71	78.38	11.67
114	78.62	45.40	53.02	820.21	357.44	52.41	95.63	11.46
115	72.24	46.16	47.44	821.99	332.52	48.61	93.05	9.69
MEAN	74.11	37.03	50.52	814.40	343.20	45.87	78.15	10.79
M_1*	69.76	34.25	47.78	809.14	325.55	37.61	70.75	9.76
M_2**	78.47	39.82	53.26	819.64	360.77	54.14	85.54	11.82

*M_1 - 95% Confidence Interval lower limit
**M_2 - 95% Confidence Interval upper limit

Appendix Table 6 a. Raw data for rainbow trout acclimated to 18°C (fall-winter series) - physical characteristics and plasma electrolytes and water content.

FISH #	SEX	LENGTH (cm)	WEIGHT (g)	PLASMA Na^+ (mM/l)	PLASMA K^+ (mM/l)	PLASMA Cl^- (mM/l)	PLASMA H_2O (ml/l)
62	F	30.5	355.0	154.50	3.18	138.96	934.0
63	M	26.5	257.0	154.50	3.27	136.50	946.0
73	M	30.0	350.0	156.60	0.78	138.90	928.0
78	F	28.0	235.0	145.80	4.52	130.80	94 .0
79	F	30.0	367.0	154.40	2.74	132.80	938.0
82	M	29.0	312.0	153.90	2.97	134.10	932.0
83	F	30.0	34 .5	155.50	2.51	135.50	956.0
86	F	29.0	208.2	155.50	3.64	129.30	922.0
87	FF	27.0	295.5	153.70	1.65	142.40	960.0
98	M	26.5	280.5	151.80	2.12	133.80	924.0
99	-	30.0	308.0	143.50	2.45	132.70	948.0
100	M	29.5	344.0	147.50	--	126.50	930.0
101	-	27.0	257.0	148.40	6.47	125.20	960.0
106	F	30.5	330.5	162.60	2.79	136.10	924.0
107	-	24.5	160.0	127.60	4.51	111.60	924.0
108	F	29.5	309.5	142.70	2.01	136.90	936.0
109	F	29.5	304.0	159.00	3.42	134.90	934.0
118	F	29.5	281.0	147.00	4.12	137.70	934.0
MEAN		28.7	293.4	150.72	3.12	132.56	936.8
M_1*		27.8	265.5	146.81	2.44	128.97	930.6
M_2**		29.5	321.3	154.62	3.79	136.15	942.9

Appendix Table 6 b. Raw data for post-opercular muscle of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
62	15.26	149.11	7.78	764.83	45.62	11.42	207.12	2.01
63	17.16	144.97	8.61	767.43	51.93	12.77	202.37	2.13
73	12.63	151.79	7.17	767.83	50.03	6.66	211.41	0.31
78	18.32	151.82	9.64	779.11	57.85	13.71	210.13	2.71
79	15.77	144,71	8.75	763.14	57.43	9.78	204.83	1.59
82	21.07	153.00	7.16	774.29	43.65	19.64	209. 3	1.79
83	13.83	156.32	7.69	761.43	50.16	8.59	219.61	1.26
86	14.46	153.39	7.72	767.95	48.06	9.71	212.83	2.09
87	17.34	146.69	9.39	776.03	61.39	10.80	205.12	0.91
98	---	156.19	---	771.77	---	---	---	---
99	12.96	144.04	7.81	774.86	51.01	7.79	198.28	1.47
100	14.27	147.56	7.56	766.62	---	---	---	---
101	12.56	148.84	7.12	764.55	33.55	10.37	203.31	3.99
106	13.32	150.43	7.82	781.32	48.23	7.47	205.02	1.71
107	14.39	---	7.39	---	---	---	---	---
108	13.98	148.13	6.84	769.43	43.55	10.69	203.95	1.21
109	14.71	146.58	7.66	771.09	45.07	10.39	201.68	2.18
118	13.08	143.72	7.46	770.68	39.17	10.00	196.25	2.82
MEAN	15.00	149.25	7.86	770.10	48.50	10.65	206.08	1.88
M_1*	13.82	147.18	7.45	767.27	44.43	8.93	202.80	1.39
M_2**	16.19	151.32	8.26	773.01	52.46	12.38	209.36	2.36

Appendix Table 6 c. Raw data for mid-dorsal muscle of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell} (mM/l cell H_2O)	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell} (mM/l cell H_2O)
62	12.41	149.43	6.59	758.26	36.81	9.32	206.96	2.05
63	13.99	149.23	7.17	754.93	41.57	10.61	208.99	2.09
73	15.43	160.59	6.59	760.06	45.93	11.52	224.82	0.29
78	15.87	156.34	--	773.26	---	---	---	--
79	12.50	152.45	6.74	755.36	42.25	8.38	213.62	1.56
82	11.37	153.72	6.32	756.62	37.24	8.52	210.62	1.79
83	18.45	145.75	6.32	761.10	39.03	17.23	203.11	1.43
86	14.11	---	6.95	766.49	---	---	---	---
87	17.38	175.66	7.53	770.70	48.95	13.66	243.26	0.78
98	---	167.79	6.58	768.84	42.95	---	233.25	0.97
99	13.45	162.50	6.60	778.14	43.44	10.00	220.74	1.34
100	13.31	153.71	6.29	766.98	---	---	---	---
101	10.49	55.89	5.46	772.07	19.71	10.06	207.03	3.98
106	11.53	165.38	6.17	777.66	36.57	7.54	223.04	1.61
107	13.54	125.57	6.76	---	---	---	---	---
108	12.77	159.64	5.86	761.57	36.04	10.51	219.93	1.14
109	12.57	161.39	6.04	766.55	33.73	9.83	219.82	2.03
118	11.15	146.29	6.06	772.95	28.15	9.41	196.26	2.93
MEAN	13.57	156.19	6.43	770.10	37.86	10.47	216.53	1.72
M_1*	12.47	151.34	6.22	767.27	37.83	8.26	209.34	1.18
M_2**	14.68	160.85	6.79	773.01	43.08	12.09	223.22	2.25

*M_1 = M_1 muscle - fall-winter series
**M_2 = M_2 muscle - fall-winter series

Appendix Table 6 d. Raw data for gonial muscle of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl/K}$ (ml/kg)	Na^+_{cell}	K^+_{cell}	Cl^-_{cell}
						(mM/l cell H_2O)		
62	14.94	152.21	8.62	767.56	53.22	11.01	213.31	1.91
63	14.79	142.57	9.03	769.00	55.70	8.66	206.01	2.07
73	---	181.35	7.92	765.14	56.07	---	255.98	0.19
78	15.36	157.95	8.62	777.09	50.79	10.96	217.18	2.65
79	1.52	151.48	7.30	755.17	46.68	7.60	214.23	1.56
82	14.93	152.56	8.40	770.71	54.29	9.18	219.78	1.65
83	12.34	144.57	8.00	757.54	51.99	6.34	204.75	1.35
86	13.64	125.89	7.02	764.57	44.17	9.39	243.93	1.82
87	15.44	148.33	8.76	775.21	56.89	9.33	206.69	0.92
98	18.02	171.74	7.63	773.31	56.37	13.27	239.71	0.91
9	15.06	142.74	7.49	780.19	48.19	8.39	194.16	1.53
100	15.32	156.01	6.71	771.84	---	---	---	---
101	11.17	150.77	6.64	771.31	29.19	9.21	202.91	4.03
106	15.38	155.29	7.96	774.47	49.85	10.04	214.11	1.62
107	11.72	---	7.96	---	---	---	---	---
108	13.70	160.31	6.97	768.27	45.16	10.05	221.57	1.09
109	13.54	154.00	7.03	768.28	40.87	9.68	211.52	2.08
118	13.46	146.69	7.46	769.76	39.56	10.47	200.67	2.76
MEAN	14.14	156.12	7.76	769.17	48.60	9.56	216.62	1.76
M_1*	13.24	150.46	7.39	765.78	44.60	8.68	207.69	1.29
M_2**	15.03	161.76	8.13	772.56	52.64	10.45	225.55	2.23

Appendix Table 6 e. Raw data for cardiac muscle of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell}	Cl^-_{cell}
						(mM/l cell H_2O)		
62	48.67	51.26	40.25	815.79	260.79	15.09	90.87	7.25
63	52.34	39.57	37.89	812.42	249.82	24.43	68.84	6.74
73	59.67	46.01	33.50	808.14	217.06	43.33	77.55	5.67
78	50.60	46.42	40.45	785.18	274.15	32.54	88.41	7.92
79	54.28	37.34	37.82	810.10	256.31	26.60	66.16	6.85
82	42.78	33.94	33.84	818.87	227.11	13.23	56.21	5.72
83	50.87	45.15	35.54	810.25	236.06	25.32	77.68	6.19
86	47.65	22.46	36.07	781.39	251.07	16.21	40.63	6.80
87	49.83	24.49	37.34	818.58	235.99	23.27	41.37	6.41
98	47.88	48.41	33.48	810.11	243.39	19.29	84.51	5.91
99	43.25	31.09	34.58	812.69	235.42	16.40	54.42	5.99
100	46.39	55.63	31.07	806.49	221.05	23.62	---	5.31
101	41.61	34.81	32.89	800.29	236.43	11.57	59.01	5.83
106	45.00	38.88	33.76	809.76	223.25	14.83	65.23	5.76
107	---	42.18	---	---	---	---	---	---
108	44.82	35.25	36.54	806.31	240.22	18.62	61.42	6.45
109	44.18	36.57	36.17	793.89	241.31	10.52	64.69	6.55
118	47.26	33.65	36.78	804.92	240.39	21.12	57.85	6.52
MEAN	48.06	39.12	35.76	806.20	240.60	20.94	65.93	6.34
M_1*	45.69	34.77	34.44	800.75	232.98	16.70	57.96	6.00
M_2**	50.44	43.47	37.08	811.64	248.17	25.19	73.90	6.68

Appendix Table 6 f. Raw data for liver of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Na}$ (ml/kg)	Na^+_{cell}	K^+_{cell}	Cl^-_{cell}
						(mM/l cell H_2O)		
62	26.97	117.36	56.28	740.26	185.31	---	210.42	55.03
63	33.60	----	52.53	733.43	230.86	---	---	41.82
73	32.59	96.76	51.43	743.37	261.09	---	200.21	31.44
78	32.91	143.26	50.62	767.35	2 9.62	---	269.41	35.64
79	34.44	122.33	51.49	729.23	236.29	---	244.17	39.89
82	33.35	118.24	54.08	734.15	230.04	---	253.29	45.97
83	35.65	127.12	46.79	735.71	245.91	---	269.29	27.49
86	38.10	118.62	56.53	744.53	260.10	---	242.91	38.39
87	32.83	117.39	54.73	756.75	261.28	---	236.06	35.37
98	35.29	174.89	48.64	748.49	250.29	---	249.62	33.39
99	42.97	96.73	45.19	755.08	347.88	---	230.48	7.21
100	38.15	113.13	41.52	754.12	274.94	---	---	14.17
101	33.96	104.64	42.06	762.47	248.95	---	198.72	22.41
106	34.42	125.59	50.52	757.29	254.78	---	235.42	37.44
107	28.58	95.59	39.76	---	238.77	---	---	---
108	35.15	101.33	52.51	741.76	261.49	---	202.94	34.38
109	36.31	102.63	53.09	737.79	241.42	---	230.41	30.27
118	---	120.40	44.66	731.86	---	---	---	---
MEAN	35.10	115.53	49.72	747.13	242.90	---	231.24	35.19
M_1*	33.18	109.64	46.13	741.51	234.21		219.44	26.12
M_2**	37.02	121.41	51.31	752.74	251.59		243.03	38.83

M_1* = 95% confidence interval lower limit
M_2** = 95% confidence interval upper limit

Appendix Table 6 g. Raw data for spleen of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
62	22.33	76.96	49.78	780.70	152.55	---	122.79	44.80
63	26.11	71.85	48.98	775.96	179.40	---	119.43	41.06
73	26.65	116.38	48.28	785.63	190.31	---	195.82	39.57
78	22.69	125.37	45.63	748.38	169.21	---	215.59	42.69
79	21.55	100.62	49.46	795.11	148.17	---	169.11	49.07
82	23.09	87.85	50.97	765.96	153.22	---	144.02	48.81
83	22.51	94.79	50.65	778.93	189.76	---	160.14	42.89
86	23.69	96.64	48.55	741.57	161.73	---	165.65	47.67
87	21.41	89.95	47.20	771.66	142.87	---	152.84	41.91
98	16.91	81.67	45.30	751.49	118.25	---	138.58	48.42
99	22.69	104.03	---	---	167.85	---	---	---
100	26.07	79.53	48.73	770.53	187.88	---	---	37.71
101	26.45	75.17	48.26	770.20	189.21	---	127.28	42.29
106	27.75	103.02	46.62	758.25	181.17	---	177.64	38.06
107	---	99.81	40.73	771.66	---	---	---	---
108	17.26	84.81	53.12	786.31	128.40	---	128.52	54.09
109	15.39	79.64	47.48	774.66	102.75	---	118.00	50.04
118	24.69	117.77	59.81	722.89	178.30	---	214.91	28.02
MEAN	23.11	94.24	47.54	764.80	161.40	---	156.09	43.53
M_1*	21.19	86.42	45.79	755.93	148.21		137.67	40.20
M_2**	25.03	102.06	49.28	773.55	174.51		174.51	46.87

Appendix Table 6 h. Raw data for gut of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
62	49.38	46.79	46.69	796.04	302.53	---	92.86	9.46
63	56.13	51.16	49.14	753.53	324.00	---	116.56	11.44
73	59.22	63.21	56.74	776.90	367.65	---	153.75	13.86
78	72.76	---	65.65	761.17	44 .92	---	---	20.75
79	55.68	52.92	54.08	762.27	366.51	---	131.18	13.66
82	48.72	51.99	50.21	770.29	336.98	---	117.48	11.57
83	57.28	59.41	61.52	735.42	407.29	---	178.19	18.69
86	63.61	32.41	66.12	786.32	460.23	---	94.25	20.28
87	59.72	40.52	61.38	776.43	387.93	---	103.68	15.80
98	50.66	39.29	46.91	740.05	341.02	---	96.65	11.76
99	68.73	28.02	71.21	756.61	484.79	---	98.71	26.19
100	60.71	26.48	57.47	776.27	408.88	---	---	15.64
101	48.14	30.57	52.59	755.12	378.04	---	74.58	13.95
106	73.26	31.74	67.44	739.35	445.97	---	103.95	22.99
107	50.49	29.58	60.64	802.25	489.03	---	87.39	19.36
108	60.56	39.95	67.48	762.49	445.62	---	122.49	21.16
109	70.56	35.02	66.59	742.82	444.26	---	112.44	22.30
118	65.73	42.75	68.31	740.17	446.47	---	139.29	25.26
MEAN	59.26	41.31	59.44	763.01	404.45	---	113.97	17.34
M_1*	55.28	35.49	55.47	753.09	376.37		99.82	14.89
M_2**	63.31	47.13	63.42	772.93	432.53		128.11	19.80

Appendix Table66 i. Raw data for brain of rainbow trout acclimated to 18°C (fall-winter series).

FISH #	Na^+ (mM/kg)	K^+ (mM/kg)	Cl^- (mM/kg)	H_2O (ml/kg)	$H_2O^{ecs}_{Cl}$ (ml/kg)	Na^+_{cell}	K^+_{cell} (mM/l cell H_2O)	Cl^-_{cell}
62	77.37	40.68	46.62	804.71	302.06	61.08	79.02	9.28
63	80.81	29.45	47.45	798.70	317.66	66.84	58.42	9.76
73	73.69	38.34	50.28	820.75	323.03	44.87	77.45	10.33
78	---	45.49	53.83	828.29	364.81	---	95.83	11.77
79	79.15	46.68	53.91	812.21	369.35	50.89	102.22	12.97
82	74.43	35.94	50.22	813.71	337.05	47.55	73.29	10.53
83	74.67	47.27	51.67	---	343.19	---	---	---
86	73.25	42.02	48.45	808.73	327.24	45.19	83.43	10.28
87	80.16	25.09	52.55	815.20	357.13	60.26	50.80	10.83
98	77.63	25.68	42.33	816.99	314.08	52.83	48.34	9.97
99	78.92	30.47	46.64	804.98	317.53	54.97	40.39	9.57
100	78.71	28.94	54.29	806.62	364.96	42.19	---	12.96
101	72.03	28.72	49.82	796.45	358.13	43.09	59.21	11.57
106	74.03	43.66	50.68	817.80	344.26	57.04	90.95	11.70
107	57.64	30.93	40.78	---	328.87	---	---	---
108	69.03	42.87	42.56	810.67	270.79	54.82	79.59	8.02
109	78.19	30.78	46.69	795.48	311.49	59.73	67.11	9.69
118	57.28	24.41	52.51	808.08	340.88	43.73	39.51	9.46
MEAN	73.01	34.62	48.72	809.78	338.40	50.83	69.51	10.19
M_1*	69.17	29.89	46.57	805.53	321.07	46.14	58.45	9.47
M_2**	76.86	39.35	50.87	814.13	355.73	55.52	80.57	10.91

* = 95% Confidence interval lower limit
** = 95% Confidence interval upper limit

Appendix VI

Appendix VI: Summary of regression analyses of plasma potassium against E_K for all tissues, and against cell Cl^- for skeletal muscle. Shown is the number of pairs (x,y) regressed (N), the 0 and 1 degree coefficient (for $y = mx + b$), the correlation coefficient and the standard error of the estimate. ('fall-winter' series)

		N	0 Degree Coefficient	1 Degree Coefficient	Coefficient of Correlation	Standard Error
Post-opercular Muscle						
pK vs E_K	@ 2°C	24	140.94	-15.35	0.9531	3.27
	@10°C	20	145.75	-15.44	0.9605	3.69
	@18°C	18	134.60	-8.69	0.9330	4.64
pK vs Cl_{cell}	@ 2°C	24	-0.17	0.52	0.9937	0.28
	@10°C	20	-0.31	0.74	0.7789	0.07
	@18°C	18	-0.15	0.65	0.9928	0.11
Mid-dorsal Muscle						
pK vs E_K	@ 2°C	21	136.77	-11.58	0.6891	7.13
	@10°C	20	145.75	-15.44	0.9772	2.77
	@18°C	18	137.01	-9.12	0.9289	5.01
pK vs Cl_{cell}	@ 2°C	21	-0.17	0.69	0.9950	0.05
	@10°C	18	-0.26	0.71	0.9892	0.06
	@18°C	18	-0.26	0.69	0.9874	0.15
Caudal Muscle						
pK vs E_K	@ 2°C	25	141.54	-15.04	0.9407	3.66
	@10°C	18	147.54	-15.97	0.9642	3.56
	@18°C	18	136.77	-9.09	0.9180	5.32
pK vs Cl_{cell}	@ 2°C	24	-0.16	0.63	0.9925	0.08
	@10°C	18	-0.31	0.73	0.9830	0.07
	@18°C	18	-0.27	0.67	0.9861	0.15
Cardiac Muscle						
pK vs E_K	@ 2°C	24	120.87	-20.65	0.8645	6.97
	@10°C	18	119.23	-14.10	0.8428	6.58
	@18°C	16	105.36	-8.72	0.8348	7.79
Liver						
pK vs E_K	@ 2°C	22	145.46	-17.24	0.9314	3.89
	@10°C	17	149.63	-15.06	0.9511	4.17
	@18°C	14	136.83	-8.48	0.9515	3.90
Spleen						
pK vs E_K	@ 2°C	24	139.52	-17.77	0.9011	5.01
	@10°C	18	139.25	-14.72	0.8786	6.89
	@18°C	16	127.60	-8.84	0.8485	7.69
Gut						
pK vs E_K	@ 2°C	25	123.88	-13.47	0.7625	7.77
	@10°C	20	137.40	-15.42	0.8393	8.43
	@18°C	16	125.95	-10.96	0.9163	6.49
Brain						
pK vs E_K	@ 2°C	23	123.88	-13.47	0.7620	7.77
	@10°C	19	121.18	-14.89	0.8830	6.26
	@18°C	15	106.28	-8.66	0.7964	9.15

Appendix VI b: Summary of regression analyses of plasma potassium against E_K for all tissues and against cell Cl^- for skeletal muscle. Shown is the number of pairs (x,y) regressed (N), the 0 and 1 degree coefficients (for y = mx + b), the correlation coefficient and the standard error of the estimate. (summer series)

		N	0 Degree Coefficient	1 Degree Coefficient	Coefficient of Correlation	Standard Error
Post-opercular Muscle						
pK vs E_K	@ 2°C	10	125.91	-9.99	0.9462	3.83
	@10°C	14	130.73	-9.84	0.9702	2.98
	@18°C	15	131.39	-10.08	0.7376	6.51
pK vs Cl_{cell}	@ 2°C	12	-0.16	0.84	0.9816	0.18
	@10°C	14	-0.14	0.75	0.9951	0.09
	@18°C	15	-0.07	0.72	0.8649	0.29
Mid-dorsal Muscle						
pK vs E_K	@ 2°C	12	127.64	-10.62	0.9467	3.94
	@10°C	12	142.45	-11.85	0.9867	1.33
	@18°C	16	137.21	-12.52	0.9233	3.71
pK vs Cl_{cell}	@ 2°C	12	-0.11	0.84	0.9875	0.14
	@10°C	12	-0.27	0.85	0.9956	0.04
	@18°C	16	-0.09	0.78	0.9252	0.23
Caudal Muscle						
pK vs E_K	@ 2°C	12	129.34	-11.77	0.9389	4.37
	@10°C	12	138.97	-16.01	0.9384	2.58
	@18°C	16	138.11	-12.39	0.9233	3.67
pK vs Cl_{cell}	@ 2°C	12	-0.24	0.83	0.9768	0.19
	@10°C	12	-0.22	0.82	0.9245	0.15
	@18°C	16	-0.28	0.79	0.8898	0.29
Cardiac Muscle						
pK vs E_K	@ 2°C	12	117.77	-9.29	0.8509	6.44
	@10°C	14	116.65	-7.29	0.7651	7.37
	@18°C	13	137.87	-16.32	0.8305	7.66
Liver						
pK vs E_K	@ 2°C	12	115.29	-7.60	0.7450	8.68
	@10°C	12	141.79	-15.52	0.9360	3.01
	@18°C	14	134.58	-11.75	0.8903	4.60
Gut						
pK vs E_K	@ 2°C	10	118.01	-11.65	0.7849	7.68
	@10°C	13	135.99	-18.54	0.6542	10.19
	@18°C	12	124.56	-13.50	0.7696	8.16
Brain						
pK vs E_K	@ 2°C	8	127.88	-11.05	0.8582	6.64
	@10°C	12	123.02	-8.23	0.8909	5.31
	@18°C	11	145.55	-14.75	0.8766	6.21